ROBUST SUMMARIES FOR
2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate

1.0 GENERAL INFORMATION

1.1. CAS NUMBER 1563-66-2

1.2. CHEMICAL NAME 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate

2.0 PHYSICAL AND CHEMICAL DATA

2.1 MELTING POINT

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical grade and purified grade

Method: Unknown

GLP: No

Year: Unknown

Results: 153-154°C (pure compound), 150-152°C (technical grade)

Data Quality: 4

References: Unknown

2.2 BOILING POINT

Remarks: Thermally decomposes at elevated temperatures (see section 2.2.1 for details).

2.2.1 THERMAL DECOMPOSITION

Test Substance: Carbofuran Technical, Purified Carbofuran Standard, Furadan 15 G (granular product)

Method: Pyrolysis followed by analysis of trapped products by HPLC with fluorescence detection (ref. 1, 2) and vapor phase infrared spectrometry (ref.2)

GLP: No

Year: 1986 and 1987

Results: The onset of thermal decomposition, in air, begins slowly just above 100°C becoming more pronounced by 130°C. At temperatures above 130°C thermal decomposition will be more readily evident. This will of course be somewhat a function of the heating rate, hence in a short time frame melting point experiment the melting point of carbofuran, which is generally greater than 150°C, can still be observed for the technical chemical. Due to decomposition a boiling point is not observable.

Data Quality: 2

References: (1) M. Alvarez to L. Masters, "Carbofuran: Pyrolysis and Combustion", October 8, 1986

(2) M. Alvarez to T. J. Clark, (Carbofuran Pyrolysis and Combustion", January 29, 1987

2.3 VAPOR PRESSURE

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate 99.9%

Method: Gas Saturation Technique

GLP:	Yes
Year:	1989
Results:	6 x 10^{-7} mm Hg (8 x 10^{-5} Pa) @ 25°C
Data Quality:	1a
References:	M. Alvarez, "Analytical Support of Carbofuran Vapor Pressure Determination", Study No. 078AF88121, January 20, 1989.

2.4 PARTITION COEFFICIENT

Test Substance:	Radio labeled 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Method:	equilibration followed by centrifugation, analysis via liquid scintillation counting
GLP:	No
Year:	1975
Results:	logP= 1.2-1.4
Data Quality:	2
References:	E. G. Brandau, "Determination of Partition Coefficients for Carbofuran, FMC 33297, FMC 25213, Certain Potential Metabolites and Two Benchmark Chemicals", Report No. M-3779, December 8, 1975

2.5 WATER SOLUBILITY

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (99.5%)
Method:	Equilibration followed by centrifugation with subsequent HPLC analysis
GLP:	Yes
Year:	1987/1998
Results:	351 ppm @ 25°C
Data Quality:	1a
References:	M. Alvarez, "Carbofuran: Evaluation of Physical Properties: Part A", Study No. 378AF8765, September 17, 1998

3.0 ENVIRONMENTAL FATE AND PATHWAY

3.1 PHOTODEGRADATION

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Method:	Following EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-2
Type:	aqueous photodegradation
GLP:	Yes
Year:	1988/1989
Light Source:	natural sunlight
Light Spectrum:	natural sunlight
Relative Intensity:	Sunlight at 38°N latitude (58 to 22,222 $\mu W/cm^2$)
Spectrum of substance:	190-299 nm (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate in acetonitrile)

Method Procedure:	An aqueous photolysis study was conducted with ^{14}C-2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate labeled in the phenyl ring at a concentration of 20 ppm in a sterile buffer solution at pH 5. The samples were exposed to natural sunlight in a water bath at approximately 25 °C along with control samples wrapped in aluminum foil. Duplicate irradiated and control samples were analyzed at 0, 3, 6, 12, 20 and 31 days after treatment. Ethylene glycol and 10% NaOH were used to trap volatile organic compounds and CO_2, respectively. Air was drawn through both the irradiated and control sample tubes into separate sets of traps. All samples were analyzed directly by LSC and HPLC.
Results:	The average mass balance for all samples was 97.1%. 7-Phenol and CO_2 were the only degradation products observed. 7-Phenol reached a maximum of 3.7% of applied and CO_2 reached a maximum of 0.3% of applied in the irradiated samples. The extrapolated half-life of photolysis was 1200 days. Extrapolated to summertime daylight conditions, the half-life of photolysis was 450 days. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was also found to slowly degrade in the dark to 7-phenol and CO_2 with a half-life of 2100 days. The formation of 7-phenol in both the irradiated and dark control samples suggests that 7-phenol is not photochemically derived. It can be concluded that 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was photolytically stable in an aqueous solution.
Data Quality:	The photolysis study is assigned a reliability code of 1a.
References:	McGovern, P, Shepler, K., "Degradation Study: Aqueous Solution Photolysis of [^{14}C] Carbofuran in Natural Sunlight at pH5", Unpublished study for FMC Corporation, Agricultural Products Group, Princeton, NJ, 1989.

3.2 STABILITY IN WATER (HYDROLYSIS)

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
Method:	not specified in the report
GLP:	no
Year:	1974
Results:	The aqueous hydrolysis of ^{14}C-phenyl 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was studied in aqueous solutions buffered to pH 5, 7 and 9 at a treatment rate of 2 ppm. Under acidic conditions at pH 5 (28°C), 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was hydrolytically stable over the 28 day test period. Under neutral conditions at pH 7 (28°C), hydrolysis occurred and the calculated half-life was 26 days. Under basic conditions at pH 9 (26°C), hydrolysis was rapid and the calculated half-life was 12 hours, with only 20% 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate remaining after 1 day. Analysis of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate in a pH 9 solution stored at 5°C showed a reduced rate of hydrolysis with a half-life of 1.5 days. The hydrolysis product formed was 7-phenol.
Remarks:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate degrades in water by hydrolysis under neutral and basic conditions but is stable under acidic conditions. Assuming that water in sewage treatment plants is either neutral or alkaline, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate will degrade.
Data Quality:	The hydrolysis study is assigned a reliability code of 2c.
References:	Cook, R.F., Robinson, R.A., 1974, Carbofuran - Hydrolysis Study. Unpublished study by FMC Corporation, Agricultural Products Group, Middleport, NY, 1974.

3.3 TRANSPORT/DISTRIBUTION (FUGACITY MODEL)

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate

Method: Estimated by EPI Suite Program (v.3.11)

Inputs: Molecular weight: 221.26
Water solubility: 351 mg/L
Vapor Pressure: 6E-007 mm Hg
Log Kow: 1.40

Year: 2004

GLP: No

Results: Distribution using Level III Fugacity model

	Mass Amount	Half-Life	Emissions
	(percent)	(hr)	(kg/hr)
Air	0.0192	9.873.43	1000
Water	39.9	900	1000
Soil	60	900	1000
Sediment	0.0912	3.6e+003	0

Persistence Time: 845 hr

Data Quality: The fugacity calculation by an acceptable method is assigned a reliability code of 2f.

References: Syracuse Research Corporation, Syracuse NY

Description of EPI-WIN Fugacity Model (Help File Excerpt):

EPIWIN v3 contains a Level III fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers (Mackay et al., 1996a, 1996b; Mackay 1991). The model in EPIWIN v3 is a direct adaptation of this methodology and approach. While it uses the same equations as Mackay's EQC Level III Fugacity Model, it was adapted specifically for use in EPIWIN. It uses exactly the same default values as the Mackay model (Note: an executable version of Mackay's EQC model can be downloaded from The Environmental Modeling Centre (Trent University) Internet web-site: http://www.trentu.ca/academic/aminss/envmodel/models.html).

A detailed description of Level I, II and III fugacity models is not presented here; please see the Mackay publications and Internet web-site cited above. In general, fugacity models predict the partitioning of an organic compound in an evaluative environment. A Level III model does not assume an equilibrium state; it only assumes steady-state. The Level III model in EPI predicts partitioning between air, soil, sediment and water using various user-input parameters and/or inputs estimated by several EPI programs.

Note: all Fugacity Half-Life Values, Emission Values, Soil Koc and Advection Values have default values or estimation methods. User intervention is not required to generate model predictions. However, more accurate user-input data (e.g. measured half-live data) should result in better model predictions. Also, modification of various default values may be required for individual evaluations. A discussion of each "Fugacity" menu selection follows.

Half-Life Values

Half-lives are required for air, soil, sediment and water ... the fugacity cannot run without them.

If the half-lives in air, water, soil and sediment are known, the "Use Half-Lives Entered Below" should be selected and the known values should be entered in the appropriate fields. Often, however, this data is not available and requires estimation. The BIOWIN and AOPWIN programs are used to make these estimates. The AOPWIN air estimate is based upon estimated hydroxyl radical and ozone rate constants. AOPWIN does have an experimental database containing more than 700 compounds. If an entered structure has a database match, the database value is used instead of the program estimate.

The water, soil and sediment half-lives are based upon BIOWIN prediction times for either ultimate or primary biodegradation. The prediction times range from "Hours" to "Recalcitrant". Each "time-range" has a default half-life value; these default values can be changed if desired. The default values were derived by Dr. Robert S. Boethling of the U.S. EPA based upon the methodology reported in the Boethling et al. (1994) journal article. The default values in EPI v3.02+ are slightly different than the default values in prior versions of EPI. If BIOWIN predicts "Weeks" for biodegradation, then a half-life of 15 days is applied to water and soil ... a half-life of 60 days is applied to sediment because the default "Half-Life Factor" for sediment is 4 times the value for water and soil (again, the default "Half-Life Factors" were derived by Dr. Robert S. Boethling). Each Biowin half-life is multiplied by the "Half-Life Factors".

The Half-life entry box contains two buttons for "Set Biowin Half-life Values". The "EPA default" button sets the values derived by Dr. Robert S. Boethling. The "Alternative" button sets slightly more conservative values.

Emission Values

The default Environmental Emission Rates are 1000 kg/hr to Air, Water and Soil (Sediment has a value of zero); these are the Mackay defaults. The Air, Water and Soil rates can be modified if desired.

EPIWIN can run the level III model once per EPI run using the emission rates shown (this is the program default) or multiple times per EPI run. Currently, "Multiple Level III Output" will run the Level III model 7 times using all permutations of Air, Water and Soil rates as either 0 or 1000 (the permutation where all rates are 0 is excluded).

Advection Values

The Advection Times apply to Air, Water and Sediment. These values should not be changed unless you are very familiar with the Mackay model. Access is available for advanced use only.

Soil Koc Value

The Fugacity Model requires a soil Koc value. By default, the Mackay Model calculates the soil Koc from the log Kow value. If desired, the soil Koc can be estimated by the PCKOCWIN program or directly entered by the user.

Other Input Parameters

The Fugacity Model cannot run without a vapor pressure. If the vapor pressure is not user-entered, the model uses the vapor pressure estimate by the MPBPWIN Program. If the MPBPWIN Program estimates a vapor pressure of zero (which can occur if an estimate is less than 1.00e-40 mm Hg), the fugacity model uses an assumed value of 1.00e-15 mm Hg (this value is low enough to have no sensitivity effect in the fugacity estimates). See section 5.3 concerning Henry's law constant inputs. The model also requires a log Kow value. If the log Kow is not user-entered, the model uses the value from the KOWWIN Program (an experimental database value is used if available instead of the estimate).

The Fugacity model in EPIWIN has limited user-access to many parameters in the Mackay Level III Model. For example, parameters such as rain rate, aerosol deposition, soil water runoff, and diffusion mass transfer coefficients cannot be changed by the EPIWIN user. For these parameters, EPIWIN relies solely upon the defaults values as determined by Mackay and co-workers. This greatly simplifies application of a Level III model for most users. If you understand the inter-workings of a Level III model and need access to these parameters, you can download the Mackay EQC Model from the Internet web-site listed above.

3.4 BIODEGRADATION

Remarks: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate degrades in water by hydrolysis under neutral and basic conditions but is stable in acidic conditions. Hydrolysis occurs under neutral conditions at room temperature (28 °C), with a half-life of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate of 26 days at pH 7. Hydrolysis is rapid at pH 9 (26 °C), only 20% 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate remains after 1 day. The half-life of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate at pH 9 is 12 hours. In acidic conditions at pH 5 and at room temperature (28 °C), 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate is hydrolytically stable after 28 days. Assuming that water in sewage treatment plants is either neutral or alkaline, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate will degrade.

3.4.1 AEROBIC SOIL METABOLISM

Test Substance: carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate)

Method: US EPA Pesticide Assessment Guidelines, Subdivision N, Section 162-1

Test type: closed system with volatile trapping devices

Contact time: soil samples were collected 0, 1, 3, 7 14, 30, 62, 92, 122, 181, 273, 365 days after treatment

GLP: yes

Year: 1993

Test conditions: The rate and degree of aerobic metabolism of ^{14}C-phenyl-carbofuran and its metabolites, were determined in an acidic and alkaline sandy loam soil. An acidic soil (pH 5.7) was collected in Georgia. A portion of this acidic soil was made alkaline (pH 7.7) by the addition of lime. Before dosing, the limed soil was incubated for approximately 2 months at approximately 25°C until the soil pH and microbial population had reached an equilibrium. The study was conducted using ^{14}C-carbofuran uniformly labeled in the phenyl ring. The study was conducted at two pH levels to address the different degradation rates in acid and alkaline soils.

The test system consisted of approximately 50 g of oven-dried soil in a 250-mL flask. The soil samples were fortified with ^{14}C-carbofuran at a nominal concentration of 3 ppm (equivalent to 6 lbs a.i./acre (6.7 kg ai/ha) which represents the highest single application for row crops) and incubated at 25°C ± 1°C under aerobic conditions in darkness for 365 days. The study apparatus was designed using ethylene glycol to trap organic volatiles and sodium hydroxide to trap CO_2.

Duplicate samples were analyzed on Days 0, 1, 3, 7, 14, 30, 62, 92, 122, 181, 273, and 365. In addition, a third sample was analyzed at each interval for measurement of pH and microbial population. Solutions in traps were changed and soil moisture was adjusted periodically. The samples were analyzed immediately upon collection: the population of aerobic bacteria and pH of the samples were determined, radioactivity in the traps was counted by LSC, radioactivity in the soil was extracted and analyzed by HPLC and the extracted soil was combusted. Selected extracts were also analyzed by TLC to confirm the identity of ^{14}C-carbofuran. Mass spectrometry was conducted to confirm significant metabolites (>10% TRR).

Results: The mass balance was >90% of applied radioactivity for all samples at all time points. A summary of the results is provided to follow.

Acidic Soil - Average Percent of Total Applied Radioactivity								
Day	^{14}C-Carbo-furan	3-OH-CF	3-K-7-P	3-K-CF	7-phenol	Trapped Volatiles	Soil Bound	Total
Day 0	97.49	0.06	0.16	0.18	0.04	ND	0.40	98.32
Day 1	96.10	0.19	0.31	0.31	ND	0.01	2.91	99.82
Day 3	95.06	0.13	ND	0.29	ND	0.03	4.64	100.15
Day 7	92.68	0.32	0.03	0.70	ND	0.05	5.94	99.71
Day 14	88.89	0.15	0.13	2.10	ND	0.09	8.35	99.71
Day 30	84.30	0.56	0.02	2.08	0.02	0.16	10.96	98.10
Day 62	82.72	ND	ND	2.60	ND	0.29	13.01	98.62
Day 92	74.98	0.56	ND	6.36	ND	0.55	15.00	97.45
Day 122	69.86	ND	ND	7.13	ND	0.94	20.89	98.81
Day 181	58.29	ND	ND	12.41	ND	2.52	24.59	97.80
Day 273	53.85	0.55	ND	11.41	ND	3.98	29.28	99.05
Day 365	43.58	0.63	1.91	11.14	0.33	4.96	35.41	97.95

Average Percent of Total Applied Radioactivity - Alkaline Soil								
Day	^{14}C-Carbo-furan	3-OH-CF	3-K-7-P	3-K-CF	7-phenol	Trapped Volatiles	Soil Bound	Total
Day 0	96.63	0.36	0.11	0.12	ND	ND	0.52	97.73
Day 1	93.22	0.18	0.33	0.09	0.03	0.01	3.23	97.08
Day 3	91.73	0.10	0.16	0.05	ND	0.02	7.18	99.23
Day 7	87.73	0.79	0.37	0.07	0.28	0.11	9.98	99.32
Day 14	83.00	0.92	0.77	0.13	0.05	0.25	12.98	98.08
Day 30	77.39	0.33	0.20	0.02	ND	0.61	18.00	96.54
Day 62	66.53	0.14	0.12	0.17	ND	1.67	27.48	96.11
Day 92	59.65	ND	ND	ND	0.59	3.18	29.60	93.01
Day 122	25.07	1.32	0.84	0.31	0.32	8.31	55.62	91.78
Day 181	27.14	1.32	0.14	0.22	0.38	10.97	55.95	96.10
Day 273	23.27	0.36	0.24	ND	1.08	14.11	59.23	98.27
Day 365	20.96	0.56	0.26	0.14	0.36	16.60	57.83	96.71

The pH of the acidic soil samples showed no significant change during the study and ranged from 5.2 to 5.8. The pH of the alkaline samples ranged from 7.4 to 8.0 for most samples except for 7.0 on Day 181 and 6.6 on Day 273. The microbial population remained viable and stable during the one-year period for both soil types.

The only major degradate (≥10% of the applied radioactivity) in the acidic soil extracts was 3-keto carbofuran, which reached a maximum of 12.41% of the applied radioactivity by Day 181 and then declined to 11.14% by Day 365. The structure of 3-keto carbofuran was confirmed by mass spectrometry and the structure of ^{14}C-carbofuran was confirmed by 2D-TLC. For the alkaline soil, radioactivity in the NaOH traps was confirmed to be $^{14}CO_2$ by barium chloride precipitation. No degradates exceeding 10% of the applied radioactivity were detected in the alkaline soil extracts. The other major products of degradation were soil-bound residues for both soil types. A maximum of 35.41% (Day 365) and 59.23% (Day 273) of the applied radioactivity was observed as bound residues in extracted acidic and alkaline soils, respectively. Fractionation of bound residues into humic acid, fulvic acid and humin indicated the presence of radioactivity in each of the three components of organic matter.

In the study, ^{14}C-carbofuran declined from 97.49% at Day 0 to 43.58% on Day 365 in acidic samples and from 96.63% on Day 0 to 20.96% on Day 365 in alkaline samples. The half-life of ^{14}C-carbofuran in the test system was determined using a first-order rate constant. The calculated half-life of ^{14}C-carbofuran was 321 days and 149 days for acidic and alkaline soils, respectively. The DT_{50} half-life was calculated using a bi-phasic decay model which gave values of 302 days and 117 days for the acidic and alkaline soils, respectively. Degradation of ^{14}C-carbofuran was observed in both the acidic and alkaline soils. Degradation of ^{14}C-carbofuran in the alkaline soil occurred at a rate at least two times faster than in the acidic soil.

Data Quality: 1a

References: Saxena, A, Marengo, Jose, White, Jon., "Aeroibc Soil Metabolism of 14C-Carbofuran", Unpublished study for FMC Corporation, Agricultural Products Group, Princeton, NJ, Report PC-0205, October 1994.

4.0 ECOTOXICOLOGY

4.1 ACUTE TOXICITY TO FISH

4.1.1 SOURCE #1

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given)

Method: Corresponded to OPPTS 850.1075; FIFRA 72-1, 3

Species: Bluegill sunfish (*Lepomis macrochirus*)

Test Concentration (nominal): 0, 30, 51, 86, 147 and 250 µg/L

Exposure Period: 96 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1976

Results: LC_{50} = 125.7 µg/l (C.I. 98.9–159.8)

NOEC = 51.0 µg/l

The static acute toxicity of carbofuran to the bluegill sunfish (*Lepomis macrochirus*) was conducted for 96 hours at Aquatic Environmental Sciences, Tarrytown, New York. Dates of experimental work not reported.

The test was initiated by first introducing the toxicant (five exposure levels, a control and acetone solvent control) into the test vessels, thoroughly mixing the toxicant and dilution water, and then introducing the 10 test organisms (four test replicates per concentration). The amount of solvent in the solvent control was equal to the amount applied to the highest concentration. The test vessels were 19 liter glass jars containing 15 liters of water. Loading in the bluegill sunfish test was 0.30 g/L.

Water used in the test was obtained from one of three wells on the Tarrytown site. Prior to study initiation the water was filtrated and characterized for pH (7.6), hardness of 44 mg/L as $CaCO_3$, total alkalinity of 29 mg/L as $CaCO_3$, a specific conductivity of 120 pmhos/cm. Temperature and dissolved oxygen were determined initially and at 48 hour intervals thereafter for the controls, high, medium and low toxicant concentrations, while pH was determined initially and at the end of 96 hours for the same treatments. Water temperatures were maintained at 22°C by placing the test vessels in controlled temperature water baths. Dissolved oxygen ranged from 8.0 to 8.6 mg/L initially and from 2.3 to 5.3 mg/L at the 96 hr measurement. PH ranged from 6.75 to 7.63 throughout the study.

The LC_{50} value was determined at 24, 48 and 96 hours. The LC_{50} value is based upon nominal concentrations of carbofuran technical. Behavioral observations made during the tests indicated that fish exposed to 250.0 µg/l showed muscle twitching, labored respiration and tetany. Percent mortality throughout the study is as following:

Percent Mortality

	Nominal Concentration						
	Control	Solvent Control	30.0 µg/L	51.0 µg/L	86.0 µg/L	147.0 µg/L	250.0 µg/L
24 hrs	0%	0%	0%	0%	20%	20%	70%
48 hrs	0%	0%	0%	0%	20%	60%	90%
96 hrs	0%	0%	0%	0%	20%	70%	90%

Data Quality: Code 1c

References: Aquatic Environmental Sciences. The Acute Toxicity of Carbofuran Technical Code 2843 Act 62.11,.12 to the Water Flea, *Daphnia magna* Straus, Bluegill Sunfish, *Lepomis macrochirus* Rafinesque, and the Rainbow Trout, *Salmo gairdneri.* Aquatic Environmental Sciences, Tarrytown, New York. FMC Study Number ACT 62.11,.12. (1976)

4.1.2 SOURCE #2

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given)

Method: Corresponded to OPPTS 850.1075; FIFRA 72-1, 3

Species: Rainbow trout (*Oncorhynchus mykiss*)

Test Concentration (nominal): 0, 80, 140, 245, 429 and 750 µg/l

Exposure Period: 96 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1976

Results: LC_{50} = 362.5 µg/L (C.I. 295.5–444.7)

NOEC = 140.0 µg/L

The static acute toxicity of carbofuran to rainbow trout (*Oncorhynchus mykiss*) was conducted for 96 hours at Aquatic Environmental Sciences, Tarrytown, New York. Dates of experimental work not reported.

The test was initiated by first introducing the toxicant into the test vessels (five exposure levels, a control and acetone solvent control), thoroughly mixing the toxicant and dilution water, and then introducing the 10 test organisms (four test replicates per concentration). The amount of solvent in the solvent control was equal to the amount applied to the highest concentration. The test vessels for the fish assays were 19 litre glass jars containing 15 litres of water. Loading in the rainbow trout tests was 0.25 g/L.

Water used in the test was obtained from one of three wells on the Tarrytown site. Prior to study initiation the water was filtrated and characterized for pH (7.49), hardness of 44 mg/L as $CaCO_3$, total alkalinity of 30 mg/L as $CaCO_3$, a specific conductivity of 120 pmhos/cm. Temperature and dissolved oxygen were determined initially and at 48 hour intervals thereafter for the controls, high, medium and low toxicant concentrations, while pH was determined initially and at the end of 96 hours for the same treatments. Water temperatures were maintained at 12°C by placing the test vessels in controlled temperature water baths. Dissolved oxygen ranged from 8.9 to 9.2 mg/L initially and from 4.7 to 8.0 mg/L at the 96 hr measurement. PH ranged from 6.81 to 7.53 throughout the study.

The LC_{50} value was determined at 24, 48 and 96 hours. The LC_{50} value is based upon nominal concentrations of carbofuran technical. Behavioral observations made during the tests indicated that fish exposed to 245.0 µg/1 and higher showed muscle twitching, labored respiration and tetany. Percent mortality throughout the study is as following:

Percent Mortality

	Nominal Concentration						
	Control	Solvent Control	80.0 µg/L	140.0 µg/L	245.0 µg/L	429.0 µg/L	750.0 µg/L
24 hrs	0%	0%	0%	0%	10%	10%	100%
48 hrs	0%	0%	0%	0%	10%	20%	100%
96 hrs	0%	0%	0%	0%	10%	70%	100%

Data Quality: Code 1c

References: Aquatic Environmental Sciences. The Acute Toxicity of Carbofuran Technical Code 2843 Act 62.11,.12 to the Water Flea, *Daphnia magna* Straus, Bluegill Sunfish, *Lepomis macrochirus* Rafinesque, and the Rainbow Trout, *Salmo gairdneri.* Aquatic Environmental Sciences, Tarrytown, New York. FMC Study Number ACT 62.11,.12. (1976)

4.1.3 SOURCE #3

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given)

Method: Corresponded to OPPTS 850.1075; FIFRA 72-1, 3

Species: Channel Catfish (*Ictalurus punctatus*)

Test Concentration (nominal): 0.1, 0.18, 0.32 and 0.56 ppm

Exposure Period: 96 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1967

Results: LC_{50} = 0.21 ppm (210 μg/L) (C.I. 0.16–.28 ppm)

The static acute toxicity of carbofuran to channel catfish (*Ictalurus punctatus*) was conducted for 96 hours at Industrial Bio-Test Laboratories, Inc. Dates of experimental work not reported.

Groups of ten channel catfish were exposed to carbofuran technical in reconstituted deionized water at nominal concentrations of 0.1, 0.18, 0.32 and 0.56 ppm. The test material was dispensed into the bioassay vessels as a 0.5% (w/v) solution in acetone. A solvent control group consisting of ten fish was run concurrently. As a quality check, each lot of experimental fish was challenged with a reference pesticide, p,p'-DDT in the form of a 0.05% (w/v) solution in acetone. Observations for mortality and behavior were made daily. pH and dissolved oxygen (OD) were measured in all bioassay vessels prior to introduction of the test material and on all test solutions in which mortality occurred. pH averaged at 7.36 throughout the study and OD averaged 6.34 ppm. Water hardness was not reported in the final study report.

No deaths or untoward behavioral reactions were noted among the fish in the solvent control group. Necropsy of the fish that died during the study, as well as those sacrificed at the end, did not reveal any significant gross lesions. Adverse fish behavior was not noted in the report for channel catfish. Number of dead catfish at each interval is as following:

Mortality of Catfish

	Nominal Concentration			
	0.10 ppm	0.18 ppm	0.32 ppm	0.56 ppm
24 hrs	0	2	5	9
48 hrs	0	1	4	1
72 hrs	0	0	0	---
96 hrs	0	0	0	---
Total	0	3	9	10

Data Quality: Code 1c

References: Schoenig, G. Four-Day Fish Toxicity on NIA 10242 Technical [Channel Catfish]. Industrial Bio-Test Laboratories, Inc. FMC Study Number NCT 169.61-02. (1967)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

4.2.1 SOURCE #1

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given).

Method: Corresponds to OPPTS 850.1010; FIFRA 72-2

Species: *Daphnia magna*

Test Concentration (nominal): 0, 5.6, 10, 18, 32 and 56 μg/L

Exposure Period: 48 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1976

Results: EC_{50} = 38.6 μg/L

NOEC = 10.0 μg/L

The acute toxicity of carbofuran to the daphnid, *Daphnia magna*, was conducted for 48 hours at Aquatic Environmental Sciences, Tarrytown, New York. Dates of experimental work not known.

Water used in the test was obtained from one of three wells on the Tarrytown site. Prior to study initiation the water was filtrated and characterized for pH (7.42), hardness of 44 mg/L as $CaCO_3$, total alkalinity of 20 mg/L as $CaCO_3$, a specific conductivity of 120 pmhos/cm. Temperature and dissolved oxygen were determined initially and at 48 hour intervals thereafter for the controls, high, medium and low toxicant concentrations, while pH was determined initially and at the end of 96 hours for the same treatments. Water temperatures were maintained at 17°C by placing the test vessels in controlled temperature water baths. Dissolved oxygen ranged from 6.7 to 8.5 mg/L throughout the study, with a pH ranging from 7.14 to 7.56.

The bioassay was conducted using five exposure levels (5.6, 10, 18, 32 and 56 μg/L), a control and solvent control. The amount of solvent in the solvent control was equal to the amount applied to the highest concentration. Four replicates were used for each exposure level in the invertebrate test. Approximately 15 adults with full brood chambers were isolated, and the next morning newly release instars were used in the study (number of organisms per test vessel was not reported). Stock solutions of toxicant were prepared by weighing the compound on an analytical balance and then diluting to volume, in a volumetric flask, with acetone. The tests were started by first introducing the toxicant into the test vessels, thoroughly mixing the toxicant and dilution water, and then introducing the test organisms. The test vessels were 250 ml beakers containing 200 ml of solution. Criteria for determining an effective concentration (EC) was immobilization. Daphnids that showed feeble swimming behaviour and slight movement of the antennae were considered immobilized. Percent mortality throughout the study is as following:

Percent Mortality

	Nominal Concentration						
	Control	Solvent Control	5.6 µg/L	10.0 µg/L	18.0 µg/L	32.0 µg/L	56.0 µg/L
24 hrs	0%	0%	0%	0%	0%	0%	55%
48 hrs	0%	0%	0%	0%	5%	20%	90%

Data Quality: Code 1c

References: Aquatic Environmental Sciences. The Acute Toxicity of Carbofuran Technical Code 2843 Act 62.11,.12 to the Water Flea, *Daphnia magna* Straus, Bluegill Sunfish, *Lepomis macrochirus* Rafinesque, and the Rainbow Trout, *Salmo gairdneri.* Aquatic Environmental Sciences ,Tarrytown, New York. FMC Study Number ACT 62.11,.12. (1976)

4.2.2 SOURCE #2

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given)

Method: Corresponds to OPPTS 850.1010; FIFRA 72-2

Species: *Daphnia magna*

Test Concentration (nominal): 10, 18, 32, 56 and 100 μg/L

Exposure Period: 48 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1981

Results: LC_{50} = 29 μg/L (C.I. 18–56)

NOEC = 18 μg/L

The static acute 48-hour toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate to the daphnid, *Daphnia magna*, was conducted on May 15 to 17, 1982, at Analytical Bio-Chemistry Laboratories Inc, Columbia, Missouri.

Ten Daphnia magna (first-instar less than 24 hours old) were randomly distributed to five concentrations in duplicate of the test. Survival of the organisms was recorded at 24 and 48 hours. Water quality parameters of temperature (20°C), water hardness (255 ppm), alkalinity (368 ppm), dissolved oxygen (8.2 to 8.4) and pH (range 7.8-8.3) were measured at time 0 and 48 hours of the study. Percent mortality throughout the study is as following:

Percent Mortality

	Nominal Concentration					
	Control	10.0 µg/L	18.0 µg/L	32.0 µg/L	56.0 µg/L	100.0 µg/L
24 hrs	0%	0%	0%	30%	100%	100%
48 hrs	0%	0%	0%	65%	100%	100%

Data Quality: Code 1c

References: Boudreau, P. et al. Static Acute Toxicity of FMC 10242 to *Daphnia magna*. Analytical Bio-Chemistry Laboratories Inc, Columbia, Missouri, USA. FMC Study Number: A81-540-01. (1981)

4.2.3 SOURCE #3

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96.1% purity)

Method: OPPTS 850.1045, FIFRA 72-3

Species: Pink shrimp (*Penaeus duorarum*)

Test Concentration (mean measured): < 4.7, 5.09, 10.5, 18.4, and 33 µg/L

Exposure Period: 96 hours

Analytical Monitoring: Yes

GLP: Yes

Year: 1987

Results: LC_{50} = 7.31 µg/L (C.I. 5.92-9.02)

The acute 96-hour flow-through saltwater toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate to the pink shrimp (*Penaeus duorarum*) was conducted at Environmental Science and Engineering, Inc. in Gainsville, Florida on July 14 to 18, 1987.

Juvenile pink shrimp were obtained from commercial supplier (AquaTek) and maintained for 4 days. During the 48 hour period immediately prior to test initiation, natural seawater was filtered to approximately 20 ppt salinity, and water temperature was 24-25°C.

The test system was a solenoid diluter with a dilution factor of 0.6. The system consisted of seven duplicate sets of glass aquaria designed to maintain approximately 9 L of test solution or dilution water. The diluter was calibrated volumetrically and equilibrated for one day prior to initiation of the test. Five 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate concentrations, a natural seawater control and an acetone solvent control were utilized. Flow rate to each duplicate aquarium provided 10.3 daily volume turnovers. Water and ambient air temperature were regulated to maintain temperatures. A 16 hour light and 8 hour dark photoperiod was maintained.

The test was initiated when 20 shrimp per treatment were impartially distributed to the test chambers. Animal loading was 0.16 g of shrimp tissue per litre of seawater passing through each aquaria in 24 hours. During testing, the shrimp were not fed. Water samples were collected from the controls and all treatments on day 0, 2 and 4 to monitor actual exposure. Water quality parameters throughout the study were: temperature (22-23°C), salinity (20 ppt), dissolved oxygen (≥6.8 ppm) and pH (range 7.6-7.9). Percent mortality throughout the study is as following:

Percent Mortality for Pink Shrimp

	Measured Concentration						
	Control	Solvent	<4.7 µg/L	5.09 µg/L	10.5 µg/L	18.4 µg/L	33.0 µg/L
24 hrs	0%	0%	0%	0%	0%	50%	100%
48 hrs	0%	0%	0%	0%	35%	70%	100%
72 hrs	0%	0%	10%	5%	60%	100%	100%
96 hrs	0%	0%	10%	20%	80%	100%	100%

Data Quality: Code 1

References: Ward, S. Acute Toxicity of FMC 10242 Technical to the Pink Shrimp (*Penaeus Duorarum*) under flow-through Conditions. Environmental Science and Engineering, Inc, Gainsville, Florida. FMC Study Number A87-2291. (1987)

4.2.4 SOURCE #4

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96.1% purity)

Method: Corresponds to OPPTS 850.1045, FIFRA 72-3

Species: Pink shrimp (*Penaeus duorarum*)

Test Concentration (nominal): 2.5, 5, 10, 20 and 40 ppb

Exposure Period: 96 hours

Analytical Monitoring: Yes

GLP: No. When the study was performed, GLP was not required.

Year: 1985

Results: LC_{50} = 12 ppb (C.I. 9.4-14)

An acute 96-hour flow-through saltwater toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate to the pink shrimp (*Penaeus duorarum*) was conducted at Environmental Science and Engineering, Inc. in Gainsville, Florida on December 2 to 6, 1985.

Pink shrimp were obtained from commercial suppliers (Auatic Indicators and AquaTek), and maintained for 5 to 18 days. During the 48 hour period immediately prior to test initiation, natural seawater was filtered to approximately 21 ppt salinity, and water temperature was 24°C.

The test system was a solenoid diluter with a dilution factor of 0.5. The system consisted of seven duplicate sets of glass aquaria designed to maintain approximately 9 L of test solution or dilution water. The diluter was calibrated volumetrically and equilibrated for one day prior to initiation of the test. Five 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate concentrations, a natural seawater control and an acetone solvent control were utilized. Flow rate to each duplicate aquarium provided approximately 7.7 daily volume turnovers. Water and ambient air temperature were regulated to maintain temperatures. A 12 hour light and 12 hour dark photoperiod was maintained. Water quality parameters throughout the study were: temperature (20-23°C), salinity (22 ppt), alkalinity (368 ppm), dissolved oxygen (≥4.9 ppm) and pH (range 7.7-8.0).

The test was initiated when 10 shrimp were impartially distributed to each test chamber providing 20 shrimp per treatment. Animal loading was 0.20 g of shrimp tissue per litre of seawater passing through each aquaria in 24 hours. During testing, the shrimp were not fed.

Water samples were collected from the solvent control and the low, middle and high test treatments on day 0 and 4 to monitor actual exposure.

Percent mortality throughout the study is as following:

Percent Mortality

	Nominal Concentration						
	Control	Solvent Control	2.5 µg/L	5.0 µg/L	10.0 µg/L	20.0 µg/L	40.0 µg/L
24 hrs	0%	0%	0%	0%	0%	0%	0%
48 hrs	0%	0%	0%	0%	10%	15%	70%
72 hrs	0%	0%	0%	30%	30%	40%	80%
96 hrs	0%	0%	0%	35%	35%	50%	100%

Data Quality: Code 1

References: Ward, S. Acute Toxicity of FMC 10242 Technical to the Pink Shrimp (*Penaeus Duorarum*) under flow-through Conditions. Environmental Science and Engineering, Inc, Gainsville, Florida. FMC Study Number A85-1791. (1985)

4.2.5 SOURCE #5

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96.1% purity)
Method:	OPPTS 850.1025; FIFRA 72-3
Species:	Eastern Oyster (*Crassostrea virginica*)
Test Concentration (nominal):	0.39, 0.65, 1.1, 1.8, 3.0 and 5.0 ppm
Exposure Period:	48 hours
Analytical Monitoring:	Yes
GLP:	Yes
Year:	1985
Results:	EC_{50} > 5.0 ppm

The acute static marine toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate to the Eastern Oyster (*Crassostrea virginica*) embryos and larvae was conducted for 48 hours at Environmental Science and Engineering, Inc. in Gainsville, Florida on July 18 to 20, 1985.

An estimated 13,140 oyster embryos were exposed in each of three replicate test vessels to carbofuran in seawater at nominal concentrations of 0.39, 0.65, 1.1, 1.8, 3.0 and 5.0 ppm for 48-hours. A solvent control (56 µL/L acetone in seawater) and a control (seawater alone) were run concurrently. Water quality parameters (dissolved oxygen (DO), pH, temperature) were recorded at the start and end of the exposure. Values were DO range 5.3-6.6 mg/L, average pH of 8.0 and temperatures of 22.8 to 22.1°C. Water salinity was 15-35 ppt. Water hardness was not recorded in the final study report. The numbers of normally developed oyster larvae in each container were counted. The percentage reduction of normal larvae was determined by comparison of the treatments to the solvent control. Significant reductions in normally developed larvae did not occur in the concentration range tested. Water quality parameters remained within acceptable limits throughout the exposure.

Data Quality:	Code 1
References:	Ward, S. Acute Toxicity of FMC 10242 Technical to Embryos and Larvae of the Eastern Oyster (*Crassostrea virginica*). Environmental Science and Engineering, Inc, Gainsville, Florida. FMC Study Number A85-1793. (1985)

4.2.6 SOURCE #6

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98.9%)
Method:	OPPTS 850.1020; FIFRA 72-2
Species:	Gammarids (*Gammarus fasciatus*)
Test Concentration (mean measured):	1.4, 2.0, 2.6, 4.7 and 9.0 µg/L
Exposure Period:	96 hours
Analytical Monitoring:	Yes
GLP:	Yes
Year:	1994
Results:	LC_{50} = 2.8 µg/L (C.I. 2.5-3.2)
	NOEC = 2.0 µg/L

The acute toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate to the gammarids, was conducted for 48 hours at T. R. Wilbury, Marblehead, Massachusetts on November 10 to 14, 1993.

Twenty amphipods (*Gammarus fasciatus*) were exposed for 96 hours to five concentrations of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate and a control. The test was performed under static conditions. The organisms were added to the test vessels within 30 minutes of media formulation and gammarids were not fed during the test.

The number of surviving organisms and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, change in behaviour or appearance) were determined visually and recorded after 24, 28, 72 and 96 hours. Dissolved oxygen, pH, conductivity and temperature were measured and recorded daily in each test chamber.

No insoluble material was observed in any test vessel during the test. The mean measured concentrations of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate were 72 to 108% of the nominal concentration and the concentration was stable throughout the test. Nominal concentrations of the active ingredient were: 0.0 µg/L (control), 1.3, 2.2, 3.6, 6.0, and 10 µg/L. Mean measured concentrations of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate were ND (none detected at the analytical detection limit of 0.80 µg/L and control), 1.4, 2.0, 2.6, 4.7, and 9.0 µg/L.

Ninety five percent survival occurred in the control and no control sublethal effects were noted during the exposure period. At the conclusion of the test the control amphipods had an average total length of 12 mm and an average wet weight of 0.03 g (loading rate was 0.3 g/L). During the definitive toxicity test the conductivity ranged from 580 to 600 µmhos/cm (mean 590 µmhos/cm), the pH ranged from 7.6 to 7.9, temperature ranged from 17.8 to 18.9°C (mean = 18.4°C), and the dissolved oxygen concentration ranged from 5.4 to 9.3 mg/L (mean = 8.0 mg/L).

Data Quality: Code 1

References: Boeri, R. L. et al. Acute Toxicity of Carbofuran to the Gammarid, *Gammarus fasciatus*. T R. Wilbury; Marblehead, Massachusetts, USA. FMC Study Number: A93-3826. (1994)

4.3 TOXICITY TO AQUATIC PLANTS

4.3.1 SOURCE#1

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97% purity)

Method: MHU/SEMA, 1988; "Manual de Testes para Avaliacao da Ecotoxicidade de Agentes Quimicos"

Species: *Chlorella* vulgaris (isolate C211/11b) – CETESB/SP

Test Concentration: 0.56, 1.00, 1.80, 3.20, 5.60, 10.0, 18.0 mg/L

Exposure Period: 98 hours

Analytical Monitoring: N/A

GLP: No

Year: 1992

Results: Based on the data, the effective concentration of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate that causes death in 50% of a population of *Chlorella* vulgaris in 96 hours was estimated to be 3.617 mg/L. The non-observed effect concentration (NOEC) was 0.385 mg/L and the observed effect concentration was 18.0 mg/L. The data were analyzed using the F-Test 95% confidence limit and regression analysis to determine the EC_{50} (96-hr). This study was conducted by FMC Corporation by BIO-AGRI – Biotecnologia Agricola S/C Ltd., Piracicaba-SP (Brazil), concluding on 11/04/92).

The test was conducted under static conditions with seven concentrations of test substance. The initial stock solution was prepared by dissolving 1 gram of test material in 100 mL of a 7% solution of Dimethyl Sulfoxide (DMSO) to facilitate solubility and 0.1 mL of Tween 80 (a dispersant), resulting in a concentration of 1000 mg/L. The pH was the stock solution was adjusted to 7.0. Serial dilutions of 100, 10, and 1 mg/L were prepared from the initial stock solution.

Chlorella vulgaris was maintained in axenic L.C. oligo medium under controlled environmental conditions. Test solutions were prepared in 500 mL Erlenmeyer flasks, adding 90 mL of L.C. Oligo medium, closed and autoclaved fro 20 minutes at 121 ° C.

Data Quality: 2d Test procedure in accordance with national standard methods with acceptable restrictions

References: Vargas A. and Bonetti R. Toxicity of Carbofuran to *Chlorella* vulgaris. Report No. FMC20/92. BIOAGRI – biotecnologia Agricola S/C Ltda. Piracicaba – SP. FMC Study Number A2003-5693.

4.3.2 SOURCE #2

Test Substance: Carbofuran (99.0%)

Method: Corresponds to: EPA Ecological Effects Test Guidelines, OPPTS 850.5400, Algal Toxicity, Tiers I and II EPA 712-C-96-164, (Adopted: April 1996); OECD No.: 201 Algal Growth Inhibition Test (Adopted: 07 June 1984)

Species: Green algae, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum)*

Test Concentration (nominal): 0, 0.5, 1.0, 2.0, 4.0, 8.0 & 16.0 mg/l (nominal)

0, 0.41, 0.99, 1.69, 3.52, 7.12 & 13.57 mg/l (measured)

Exposure Period: 72 hours

Analytical Monitoring: Yes

GLP: Yes

Year: 2005

Results: EC_{50} = 4.99 mg/l (C.I. 4.50–5.48)

LOEC = 1.69 mg/l

NOEC = 0.99 mg/l

The static acute toxicity of carbofuran to the unicellular green alga (*Pseudokirchneriella subcapitata*) was conducted for 72 hours at LAB International Research Centre Hungary Ltd., Veszprém, Szabadságpuszta, Hungary. Dates of experimental were 11 April 2005 to 13 June 2005.

Based upon the results from a preliminary study, nominal concentrations of 0.50, 1.00, 2.00, 4.00, 8.00 and 16 mg/l carbofuran were used in the main study. For determination of the test concentration, water samples were taken from each concentration level at the start and from each testing flask at the end of the test.

The test design included three replicates at each test concentration. There were six for untreated controls and three zinc chloride (positive controls) replicates included in the study.

The algal concentration was 10^4 cells/ml in all of the test cultures, at the start of the study. 250 ml glass flasks containing 100 ml of solution were used. The stock water was prepared according to OECD 201 guideline with the appropriate volumes of The Mineral Salts Test Medium. The water used was further characterized for pH (8.42-8.60 at the start; 7.65-9.38 at the end) and temperature (21.8-23.9°C). The study was conducted under a climate controlled environment, subject to a continual light intensity of approximately 6803 lux.

The algal cell concentration of each flask was determined by microscope during the 72-hour test at 24-hour intervals. Statistical comparison of average growth rates and areas under the growth curves in untreated control and in treated groups were carried out using analysis of variance (ANOVA) and Dunnett's Test by TOXSTAT software.

The EC_{50} value was determined at 72 hours. The EC_{50} value is based upon initial measured concentrations of carbofuran technical.

Data Quality: Code 1a

References: Vertesi, A. Algal Growth Inhibition Test with Carbofuran Technical (FMC 10242) Test Item. LAB International Research Centre Hungary Ltd., Veszprém, Szabadságpuszta, Hungary FMC Study Number A2005-5903 (2005).

5.0 TOXICITY

5.1 ACUTE TOXICITY

5.1.1 ORAL

5.1.1.1 SOURCE #1

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical MRV-131 (purity not given).

Guideline: Corresponded to OPPTS 870.1100

Method: The compound was tested for acute oral toxicity at doses of 5.90, 7.78, 8.93, 10.26, 13.5 and 17.80 mg/kg body weight. The test article dissolved in corn oil was administered by gavage in a dose volume of 5 ml/kg body weight to 10 young adult rats (5 males and 5 females) per dose group.[1] The animals were observed for mortality and pharmacotoxic signs at 1, 3 and 5 hours and then for up to 14 days following dosing.

GLP: No

Year: 1979

Species/strain: Sprague-Dawley (TacN(SD)fBR) rat

Sex: Both

No. Animals/Group: 10 young adult rats (5 males and 5 females)

Vehicle: corn oil

Route of administration: oral (gavage)

Results: Signs of toxicity, including tremors, hyper-reactivity to external stimuli and piloerection appeared within the first hour after dosing and disappeared by 24 hours unless death supervened. Most deaths occurred within the first hour and most of these animals had congested lungs at necropsy. No other visible lesions indicative of systemic toxicity were observed. The treated animals that survived showed weight gains similar to that of the control groups.

Conclusion: The LD_{50} for males was 10.5 (mg/kg), females was 8.0 (mg/kg), and combined was 8.8 (mg/kg).

Data Quality: 2c

Reference: Ellemen, P.N.; Acute Oral Toxicity (FIFRA-EPA) in Rats; Cosmopolitan Safety Evaluation (CSE) Inc, Somerville, New Jersey, USA; Unpublished Report No: A79-339; 14 September 1979.

Footnote 1: 8.93 mg/kg was administered only to a group of 5 females

5.1.1.2 SOURCE #2

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%)
Guideline:	Corresponded to OPPTS 870.1100
Method:	Groups of Sprague-Dawley rats consisting of ten males and/or ten females were orally administered (by gavage) graded dosages of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical as a 0.10% (weight/volume) suspension in corn oil. Observations for toxicity were conducted at 0.5, 1, 2, 3, 4 and 6 hours on the day of dosing and twice daily thereafter for thirteen days; on day 14 they were observed once. Body weights were recorded on day 0, 7 and 14 of the study. A gross necropsy was performed on all animals.
GLP:	No
Year:	1983
Species/strain:	Sprague-Dawley rats
Sex:	Both
No. Animals/Group:	10 males and/or 10 females
Vehicle:	corn oil
Route of administration:	oral gavage
Results:	The mortality data by dose for males was 20% at 9.0 mg/kg, 30% at 12.0 mg/kg, and 80% at 17.0 mg/kg. The mortality data by dose for females was 30% at 3.0 mg/kg, 60% at 6.0 mg/kg, 60% at 9.0 mg/kg, and 80% at 12.0 mg/kg. The predominant clinical signs were tremors, decreased locomotion, abdomino-genital staining as well as oral, ocular, and nasal discharges. All signs of toxicity subsided by day 2 of the study. At necropsy, there were no internal gross lesions noted in any animal.
Conclusion:	Under the conditions of this study, the test material is classified as highly toxic to adult rats with a male LD_{50} of 13.2 mg/kg and a female LD_{50} of 5.3 mg/kg.
Data Quality:	1b
Reference:	Norvell M J (1983a); Acute Oral Toxicity of FMC 10242 Technical in Rats; FMC Toxicology Laboratory, Somerville, New Jersey, USA; Unpublished Report No: A83-1101; 3 November 1983.

5.1.1.3 SOURCE #3

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97%).
Guideline:	Corresponded to OPPTS 870.1100
Method:	Groups of Sprague-Dawley rats consisting of ten males and/or ten females were orally administered graded dosages of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical as a 0.10% (weight/volume) suspension in corn oil. Dose levels of 9.0, 13.0, 14.0, 15.0 and 17.0 were used. Observations for toxicity were conducted at 0.5, 1, 2, 3, 4 and 6 hours on the day of dosing and twice daily thereafter for thirteen days; on day 14 they were observed once. Body weights were recorded on day 0, 7 and 14 of the study. A gross necropsy was performed on all animals.
GLP:	No
Year:	1983

Species/strain:	Sprague-Dawley rats
Sex:	Both
No. Animals/Group:	10 males and/or 10 females
Vehicle:	corn oil
Route of administration:	oral (gavage)
Results:	The mortality data for males was 0% at 9.0 mg/kg, 10% at 13.0 mg/kg, 70% at 14.0 mg/kg, 90% at 15.0 mg/kg, and 90% at 17.0 mg/kg. Mortality for females included 0% at 2.0 mg/kg, 20% at 3.5 mg/kg, 50% at 5.0 mg/kg, 70% at 9.0 mg/kg, and 100% at 13.0 mg/kg. The predominant clinical signs were tremors, decreased locomotion, abdomino-genital staining, chromorhinorrhea, chromodacryorrhea and oral discharge. All signs of toxicity subsided by day 2 of the study. All surviving animals gained weight by day 14 of the study. There were no internal gross lesions noted in any animal at necropsy.
Conclusion:	Under the conditions of this study, the test material is classified as highly toxic to adult rats with a male LD_{50} of 13.3 mg/kg and a female LD_{50} of 5.6 mg/kg.
Data Quality:	1b
Reference:	Norvell M J (1983b); Acute Oral Toxicity of FMC 10242 Technical in Rats; FMC Toxicology Laboratory, Somerville, New Jersey, USA; Unpublished Report No: A83-1102; 11 April 1983.

5.1.1.4 SOURCE #4

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (85%)
Guideline:	Corresponded to OPPTS 870.1100
Method:	The purpose of this study was to evaluate the test material for its systemic toxicity when administered orally to fasted Sprague Dawley rats. The test material was formulated in corn oil to obtain a 5% weight/volume solution. The rats were fasted overnight prior to dosing. The test material was introduced directly into the stomach of each animal by means of a straight, rigid 16 gauge stainless steel dosing needle. The following dose levels/numbers of animals were used: The animals were observed at 1 and 3 hours on the day of dosing and daily thereafter until day 14. Animals were checked for mortality twice daily. Body weights were taken on days 0, 7, and 14. Animals dying intercurrently as well as those animals surviving treatment and killed on day fourteen with CO_2 gas were necropsied.
GLP:	No
Year:	1981
Species/strain:	Sprague Dawley rats
Sex:	Both
No. Animals/Group:	10 males/10 females (exception: 0 males and 10 females at 0.002 mg/kg)
Vehicle:	corn oil
Route of administration:	oral gavage
Results:	The mortality data for males was 20% at .004 g/kg, 30% at .007 g/kg, and 70% at .015 g/kg. Mortality data for females was 0% at .002 g/kg, 40% at .004 g/kg, 70% at .007 g/kg, and 80% at .015 g/kg.

All the rats which died succumbed within the first hour after dosing. Clinical signs among the rats of both sexes included tremors, oral discharge, fasciculation, decreased locomotion and swollen cheeks. These signs were dose-related and are consistent with those signs expected from a cholinesterase inhibiting agent. The rats which survived treatment gained weight.

The abnormalities noted at necropsy in the rats that died during the study included oral discharge and a white frothy material in the trachea. Gross findings in rats which survived the treatment and were killed on day 14 were not judged to be treatment related.

Conclusion: The LD_{50} for males was 10 mg/kg, for females 6 mg/kg, and the combined was 7 mg/kg.

Data Quality: 1b

Reference: Seaman L R (1981a); Acute Oral Toxicity of Furadan 85 DB in Rats; FMC Toxicology Laboratory, Somerville, New Jersey, USA; Unpublished Report No: A81-599; 30 December 1981.

5.1.2 DERMAL

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96.1%)

Guideline: Corresponded to OPPTS 870.1200 with the following exceptions:

- Intact and abraded skin used
- males/dose only except at 2010 mg/kg when 5 males and 5 females were used

Method: There were two vehicle control groups (one abraded and one intact) and seven treatment groups (four abraded and three intact) in this study at 0 (vehicle control), 1640, 2010, 2460, 3010, 3680 (mg/kg).

The test material for each animal in the treatment groups was moistened with 3.00 ml/kg of 0.9% saline and was applied under surgical gauze onto the exposure area. The vehicle control animals were prepared and wrapped in an identical manner, but only received 3.00 ml/kg of 0.9% saline.

Observations for pharmacologic and/or toxicologic effects and mortality were made on the day of treatment and at least twice daily thereafter for fourteen days (day of treatment considered Day 0). Individual body weights were recorded on Days 0, 7, and 14 or at the time of discovery after death. A gross necropsy examination was conducted on each animal at termination of the study or at the time of discovery after death.

GLP: No

Year: 1981

Species/strain: New Zealand White rabbits

Sex: males and females

No. Animals/Group: 10 (5/sex), 4/control

Vehicle: saline

Route of administration: dermal

Results: All vehicle control animals appeared normal for the duration of the study. Prominent in-life observations for the animals treated with 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate included decreased activity, ataxia, body tremors, clear nasal discharge, constricted pupils, decreased defecation, decreased urination, diarrhoea, hematuria, mucoid diarrhoea, muscle tremors, no defecation, no urination, ptosis, salivation, swollen penal sheath, and swollen testes.

	A gross necropsy examination was conducted on each animal. There were no observable abnormalities in any of the vehicle control animals. Of the gross necropsy those described as diarrhoea, lacrimation, polyuria, salivation, swollen testes, clear liquid in stomach, discoloration of the contents of the urinary bladder, discoloration of the stomach and intestinal mucosa, excessive amount of red liquid in abdominal cavity, gastrointestinal tract empty, serosal blood vessels pronounced on entire gastrointestinal tract, urinary bladder completely full, urinary bladder empty, and variations thereof were considered unusual findings and possibly related to the administration of the test material.
Conclusion:	As indicated by the data, the acute dermal toxicity for actual 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical is greater than 2000 mg/kg for abraded males, abraded females, and intact males.
Data Quality:	1b
Reference:	Mehta C S (1981); Acute Dermal Toxicity Study in Rabbits Carbofuran Technical 96.1% M607210; Stillmeadow Inc, Sugar Land, Texas, USA; Unpublished Report No: A81-564; 9 July 1981.

5.1.3 INHALATION

5.1.3.1 SOURCE #1

Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (95.6%).
Guideline:	Corresponded to OPPTS 870.1300 except that exposure was for 1 hour due to toxicity of the test compound
GLP:	Yes
Year:	1994
Method:	Five groups of Sprague-Dawley rats were exposed to a respirable aerosol of the test material at concentrations of 0.70, 0.16, 0.094, 0.062 or 0.014 mg/l. Animals were exposed for 1 hour in a nose-only exposure chamber. Gravimetric airborne test material samples were taken twice during each exposure. Particle size samples were taken once during each exposure. Observations for toxicity and mortality were performed frequently during the exposure, upon removal of the rats from the chamber, at one hour post-exposure and daily thereafter for 14 days. Individual body weights were recorded immediately prior to the exposure on day 0 and on days 7 and 14. On day 14, all animals were sacrificed and gross necropsy examinations were performed.
Species/strain:	Sprague-Dawley rats
Sex:	males and females
No. Animals/Group:	10 (5/sex)
Vehicle:	N/A
Route of administration:	Inhalation
Results:	Clinical signs noted during the study included ataxia, tremors, splayed hindlimbs, dyspnea, oral discharge, chromodacryorrhea, and nasal discharge. All of these signs resolved by the day following the exposure. All surviving animals were normal from the day 3 observations through study termination. All surviving animals gained weight during the study. All the animals which died prior to the study termination had lung discoloration at necropsy. There were no gross internal lesions noted in any animal which survived to study termination.
Conclusion:	Under the conditions of this study, Furadan 95 DB MUP had a male LC_{50} value of 0.11 mg/l, a female LC_{50} value of 0.10 mg/l and a combined LC_{50} value of 0.11 mg/l.
Data Quality:	1b

Reference:	Signorin J (1995); Furadan 95 DB MUP Dot Acute Inhalation Toxicity Study in Rats; FMC Corporation Toxicology Laboratory, Princeton, New Jersey, USA; unpublished report no: A94-4097; February 1995.

5.1.3.2 SOURCE #2

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (85%)
Guideline:	Corresponded to OPPTS 870.1300
Method:	Fifteen male and fifteen female young adult Sprague-Dawley Crl CD ® BR strain rats were randomly allocated to cages using random letter tables. A dust atmosphere was produced from the test material using a dust feeder mechanism located at the top of the exposure chamber and driven by a variable speed motor. The dust feed was connected to a metered compressed air supply. Homogeneity of the test atmosphere within the chamber was not specifically determined during this study, but, chambers of the same design have been fully validated and shown to produce evenly distributed atmospheres in the animals' breathing zone with a wide variety of test materials. Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring. Only the nose of each animal was exposed to the test atmosphere. Three groups, each of ten rats (five males and five females) were subjected to a single exposure to the test material for a period of up to four hours. Based on the expected toxicity of the test material and pre-study sighting, a target concentration of 0.05 mg/l was used for the first exposure. Further concentrations were selected after consideration of the results of the previous exposure. The particle size of the generated atmosphere of the test material inside the exposure chamber was determined at least once during each exposure period Animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days. Individual bodyweights were recorded prior to treatment on the day of exposure and on Days 7 and 14 or at death. At the end of the fourteen day observation period, the surviving animals were euthanized. All animals, including those that died or were euthanized during the study, were subjected to a gross necropsy, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.
GLP:	Yes (UK Department of Health)
Year:	1999
Species/strain:	Sprague-Dawley Crl CD ® BR rats
Sex:	Both
No. Animals/Group:	10 (5 males and 5 females)
Vehicle:	N/A
Route of administration:	Inhalation
Results:	The actual concentration of the test material was measured at intervals during each exposure period. The mean values obtained were 0.03, 0.04, and 0.07 mg/l.

The mortality data for males was 0/5 died at 0.03 mg/l, 0/5 at 0.04 mg/l, and 5/5 at 0.07 mg/l. The mortality data for females was 0/5 died at 0.03mg/l, 3/5 at 0.04 mg/l, and 5/5 at 0.07 mg/l.

On removal from the chamber following exposure to 0.03 or 0.04 mg/l surviving animals commonly showed wet fur, hunched posture, pilo-erection, fasciculations, ataxia, increased or decreased respiratory rate and red/brown staining around the eyes or snout. Several animals showed laboured respiration and there were signs of body tremors, lethargy, exophthalmos, pallor of the extremities and tiptoe gait. One hour after completion of exposure, wet fur, fasciculations, body tremors and pallor of the extremities were no longer evident

One male exposed to 0.04 mg/l showed reduced bodyweight gain during Week 1 of the study but otherwise normal bodyweight development was noted in surviving animals over the study period.

Conclusion: The acute inhalation median lethal concentrations (LC_{50}) were reported as 0.05 mg/l for all animals, 0.06 mg/l for males, and 0.04 mg/l for females.

Data Quality: 1b

Reference: Blagden S M (1999); Furadan 85 DB: Acute Inhalation Toxicity (Nose Only) Study in the Rat; Safepharm Laboratories Limited, Derby, United Kingdom; unpublished report no: 240/186; 22 February 1999; dates of experimental work: 21 December 1998 – 20 January 1999.

5.1.4 SKIN IRRITATION

Test Substance: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (85%)

Guideline: Corresponded to OPPTS 870.2500 with the following exceptions:

- Abraded and non-abraded skin were used
- Dressing remained in place for 24 hours

Method: Approximately twenty-four hours prior to test material administration the trunks of six rabbits (3 male and 3 female), weighing between 2.0 – 3.0 kg, were clipped free of hair. Two test sites, each at least 5 centimetres square, were located on each side of the spinal column. The two sites on the right were abraded and the two sites on the left were left intact.

A dose of 0.5g of test material was weighed onto an 8 ply 2 x 2 inch gauze patch and moistened with saline. A gauze patch was secured to each test site with hypoallergenic tape. Twenty-four hours later the wrapping and patches were removed and the test sites were wiped with clean gauze. Approximately 30 minutes later the four test sites were assessed for irritancy according to the Draize method. The sites were again evaluated at seventy-two hours and at day four.

The animals were weighed once on the day before dosing. Clinical signs, as noted, were recorded. Animals that died after treatment were not necropsied.

GLP: No

Year: 1981

Species/strain: New Zealand rabbits

Sex: Both

No. Animals/Group: 6 (3 male and 3 female)

Vehicle: saline

Route of administration: dermal

Results:	There were no deaths reported in the study. On day 14, two rabbits had a nasal discharge not deemed to be treatment related. The primary irritation index of the test material, based on the irritation at twenty-four and seventy-two hours was 1.2/8.0. This represents a slight degree of irritation. Abrasion of the epidermis caused only a slight increase in irritancy, (abraded 1.3/8.0, intact 1.1/8.0). Irritation score for day four was 0.0/8.0.
Conclusion:	Under the conditions of the study, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate base would not be considered to be an irritant.
Data Quality:	1b
Reference:	Seaman L R (1981b); Primary Skin Irritation Study Furadan 85 DB; FMC Toxicology Laboratory, Somerville, New Jersey, USA; Unpublished Report No: A81-598; 16 October 1981.

5.1.5 EYE IRRITATION

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (85%)
Guideline:	Corresponded to OPPTS 870.2400
Method:	Prior to instillation of the test material nine rabbits (four male, five female) were selected for study after examination of both eyes for ocular defects. The right eyes of the rabbits were used for treatment; the left eyes served as controls. Ten milligrams of the test material (water served as the solvent) was instilled into the lower conjunctival sac at a dose of 0.01 g chosen based on the toxicity of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate observed in a preliminary study. The eyes of six of the rabbits remained unwashed and the eyes of three of the rabbits were washed. The washing was affected by gentle expression of 100 ml of lukewarm tap water from syringe barrels 20–30 seconds after instillation of the test material. The rate of application of the wash water was approximately one minute. The eyes were scored for irritancy using the Draize method at 1, 24, 48 and 72 hours after dosing. After the twenty-four hour examination with a penlight type light, the eyes were examined with the aid of sodium fluorescein dye. Those animals that showed corneal defects, if any, were examined again at later assessment times.
GLP:	No
Year:	1981
Species/strain:	New Zealand Albino rabbits
Sex:	Both
No. Animals/Group:	9 (4 male and 5 female)
Vehicle:	N/A
Route of administration:	ocular instillation
Results:	The material was readily absorbed from the eye and elicited signs of systemic toxicity. The clinical signs included fasciculation, muscle weakness and lack of coordination. The maximum Draize score for unwashed test eyes (3.0/110) and washed test eyes (0.7/110) was found at the one hour reading. When assessed at 24 hours with fluorescein dye, none of the animals showed signs of dye retention indicating the surface epithelial cells of the cornea remained intact. Washing the eye with tap water shortly after exposure had a modifying effect on the eye irritation, however, it did not affect the appearance of clinical signs.
Conclusion:	Not an eye irritant.

Data Quality:	1b
Reference:	Seaman L R (1981c); Primary Eye Irritation Study Furadan 85 DB; FMC Toxicology Laboratory, Somerville, New Jersey, USA; unpublished report no: A81-601; 9 October 1981.

5.1.6 SKIN SENSITIZATION

5.1.6.1 SOURCE #1

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98.9%)
Guideline:	Corresponded to OPPTS 870.2600
Method:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical (0.5g) was applied weekly, following moistening with tap water, to ten male Hartley guinea pigs for three weeks. Fourteen days after the third induction treatment, these animals were challenged at a virgin site with 0.5g of the moistened test material. Additionally, ten naive guinea pigs also received 0.5g of the moistened test material for the challenge application. Skin reactions were recorded 24 hours following each induction, and 24 and 48 hours following challenge. Body weights were recorded at initiation and termination.
GLP:	Yes
Year:	1999
Species/strain:	Hartley guinea pigs
Sex:	Males
No. Animals/Group:	10
Vehicle:	N/A
Route of administration:	dermal
Results:	One of the naive test animals was found dead during the 24-hour scoring of the challenge application. The cause of death could not be determined, but this death was not judged to be attributable to the test material. All other animals remained healthy and gained weight during the study. No irritation was noted among any test or naive control animals at any time during the study.
Conclusion:	The test material was judged to be non-sensitising when topically applied to Hartley guinea pigs.
Data Quality:	1b
Reference:	Freeman C (1999); Skin Sensitisation Study in Guinea Pigs; FMC Toxicology Laboratory, Princeton, New Jersey, USA; Unpublished Report No: A-98-4959; 3 May 1999.

5.1.6.2 SOURCE #2

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (85%)
Guideline:	Corresponded to OPPTS 870.2600
Method:	Ten test and five control animals were used to assess the contact sensitisation potential of the test material. Shortly before treatment on Day 0 the hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal. A row of three injections (0.1 ml each) was made on each side of the mid-line. The injections were: a) Freund's Complete Adjuvant plus distilled water in the ratio 1:1 b) a 0.1% w/v formulation of the test material in distilled water

	c) a 0.1% w/v formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus distilled water. Approximately 24 and 48 hours after intradermal injection the degree of erythema at the test material injection sites (ie injection site b) was evaluated. Day 7, the same area on the shoulder region used previously for intradermal injections was treated with a topical application of the test material formulation. This occlusive dressing was kept in place for 48 hours. The degree of erythema and oedema was quantified one and twenty-four hours following removal of the patches. Any other reactions were also recorded. Controls were treated in a similar manner without the use of the test material. On treatment Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was treated with the test material at the maximum non-irritant concentration (25% w/w in distilled water) was applied to the shorn right flank of each animal. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 10% w/w in distilled water was similarly applied to a skin site on the left shorn flank. The challenge sites were swabbed with cotton wool soaked in distilled water to remove residual material. Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and edema was quantified. Any other reactions were also recorded.
GLP:	Yes (UK Department of Health)
Year:	1999
Species/strain:	Albino guinea pigs
Sex:	Males
No. Animals/Group:	10 treated, 5 control
Vehicle:	N/A
Route of administration:	dermal
Results:	Very slight erythema was noted at the intradermal induction sites of all test group animals at the 24-hour observation and in seven test group animals at the 48-hour observation. Very slight erythema was noted at the intradermal induction sites of three control group animals at the 24 and 48-hour observations. Very slight or well-defined erythema was noted at the induction sites of all test group animals at the 1-hour observation. Very slight erythema was noted at the induction sites of six test group animals at the 24-hour observation. No skin reactions were noted at the treatment sites of control group animals at the 24-hour observation. No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48-hour observations. Bodyweight gains of guinea pigs in the test group, between Day 0 and Day 24, were comparable to those observed in the control group animals over the same period.
Conclusion:	The test material produced a 0% (0/10) sensitisation rate and was classified as a non-sensitiser to guinea pig skin.
Data Quality:	1c
Reference:	Allen J D (1999); Furadan 85 DB: Magnusson & Kligman Maximisation Study in the Guinea Pig; Safepharm Laboratories Limited, Derby, United Kingdom; Unpublished Report No: 240/187; 23 March 1999.

5.1.6.3 SOURCE #3

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (purity not given)
Guideline:	Corresponded to OPPTS.870.2600 with the following exceptions: • Caging not described • 5 animals only were used in the treatment group • 10 injections of the test material were made followed by a challenge injection two weeks later
Method:	Five white male guinea pigs were used in evaluating the skin sensitising properties of the test material. Ten injections were made intracutaneously into each of the five guinea pigs. The injections were made every other day at random into an area of skin about three to four centimetres square just below the midline of the back. The first injection consisted of 0.05 ml while the remaining nine injections were 0.10 ml each. Two weeks after the tenth injection, a final challenge injection of 0.05 ml of freshly prepared solution was made into each animal. This final injection was made into the skin of the flank slightly below the original injection area. Twenty-four hours after the challenge injections, a reading of the diameter, erythema and edema of the reaction site was made. The readings were then compared with similar readings made at the sites of the first injection 24 hours after such injections had been administered.
GLP:	No
Year:	Not given.
Species/strain:	Albino guinea pigs
Sex:	Male
No. Animals/Group:	5
Vehicle:	N/A
Route of administration:	dermal
Results:	The results indicate erythema and edema following the initial injections with mean scores of 2.4 and 2.8, respectively. The erythema and edema mean scores following the challenge injections were 2.2 and 2.2, respectively.
Conclusion:	The results of this study indicate that 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical is nonsensitising to the skin of the guinea pig.
Data Quality:	2a
Reference:	Schoenig G (1967); Skin Sensitisation Test on NIA 10242 Technical; Industrial Bio-Test Laboratories, Northbrook, Illinois, USA; unpublished report no: NCT 176.36; 9 July 1981.
Footnote 1:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical was administered in the form of a 0. 1 per cent (w/v) solution in propylene glycol and the positive control (dinitrochlorobenzene) was administered in the form of a 0. 1 per cent (w/v) aqueous solution

5.2 GENETIC TOXICITY IN VITRO

5.2.1 SOURCE#1

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98.3%)
Method:	OPPTS 870.5100

Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate caused a weak positive response (2.2-fold increase) on tester strain TA1535, only in the absence of rat liver microsomes. There was no indication of activity in the presence of liver microsomes.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate would not be considered mutagenic under the conditions of this study.
Data Quality:	1b
Reference:	Haworth S R and Lawlor T E (1983a); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-868; 23 September 1983.

5.2.2 SOURCE#2

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983

Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	Negative. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not cause a positive response on any of the tester strains. A 1.9-fold dose-responsive increase in TA1535 revertants per plate was observed in the absence of rat liver microsomes. The magnitude of this increase did not meet the criteria for a positive response.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of S. typhimurium in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983b); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report no: A83-913; 23 September 1983

5.2.3 SOURCE#3

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (80%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	The results of the *Salmonella*/mammalian-microsome mutagenicity assay indicate that 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did cause a weak positive response on TA1535 (2.1x the control value), but only in the absence of rat liver microsomes.

Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence of metabolic activation. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was weakly positive in one strain in the absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983c); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-948; 12 September 1983.

5.2.4 SOURCE#4

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (99%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	Negative. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not cause a positive response on any of the tester strains. A 1.7-fold dose-responsive increase in the TA1535 revertants per plate was observed in the absence of rat liver microsomes. The magnitude of this increase did not meet the criteria for a positive response as described in the protocol.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983d); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-949; 12 September 1983.

5.2.5 SOURCE#5

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	The results indicate that under the conditions of this study, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did cause a weak positive response on TA1535 (1.97x the control number of revertants), but only in the absence of rat liver microsomes. No indication of activity was observed in the presence of rat liver microsomes.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983e); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-950; 12 September 1983.

5.2.6 SOURCE#6

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay

	Activation system: rat liver Aroclor-induced microsomes
	Media: minimal top agar with histidine and biotin
	No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	Negative. A 1.9-fold dose-responsive increase in the number of TA1535 revertants per plate was observed in the absence of rat liver microsomes. The magnitude of this increase did not meet the criteria for a positive response as described in the protocol.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of S. typhimurium in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983f); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-951; 23 September 1983.

5.2.7 SOURCE#7

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay
	Activation system: rat liver Aroclor-induced microsomes
	Media: minimal top agar with histidine and biotin
	No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the

	observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	Negative.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhim*urium in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983g); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-973; 4 October 1983. \

5.2.8 SOURCE#8

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 100, 500, 2500, 5,000 and 10,000 ug/plate
Statistical Methods:	Average and standard deviation of each triplicate plating were determined. For a test to be considered positive, the number of revertants per plate must be at least 2x for at least one tester strain. The increase in mean number of revertants per plate must be accompanied by a dose response to increasing concentration of the test article. If the observed dose-responsive increase in TA 1537 or TA 1538 revertants per plate is less than three-fold, the response must be reproduced.
Results:	Negative.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Haworth S R and Lawlor T E (1983h); *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test); Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-1063; 1 November 1983.

5.2.9 SOURCE#9

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 1, 10, 100, 500, 1,000 2,500, 5,000 and 10,000 ug/plate
Statistical Methods:	For strains TA-1535, -1537, and -1538: If the solvent control value is within the normal range, a test material producing a positive response equal to three times the solvent control value is considered mutagenic. For strains TA-98 and TA-100: : If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value is considered mutagenic.
Results:	Negative.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	DeGraff W G (1983a); Mutagenicity Evaluation of FMC 10242 (Study A83-1037) in the Ames *Salmonella*/Microsome Plate Test; Litton Bionetics Inc, Kensington, Maryland, USA; Unpublished Report No: A83-1037; August 1983.

5.2.10 SOURCE#10

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (99%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3

Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 61.7, 185.2, 555.6, 1,667, and 5,000 ug/plate
Statistical Methods:	If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value is considered mutagenic. A chemical that exhibits a positive dose response over three concentration with the smallest of these increases equal to twice the solvent control is considered to be mutagenic.
Results:	Negative. While a greater than two-fold increase in mutant colonies of TA1535 was induced at the 5,000 μg/plate concentration in the nonactivated phase of testing, a dose related effect was not uncovered. This response did not meet the criteria for a positive test (dose response over three doses with the smallest increase of at least twice the solvent control.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b.
Reference:	Farrow M G (1983a); *Salmonella typhimurium*/Mammalian Microsome Plate Incorporation Assay with Compound FMC 10242; Hazleton Laboratories America Inc, Vienna, Virginia, USA; Unpublished Report No: A83-905; 5 December 1983.

5.2.11 SOURCE#11

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Activation system: rat liver Aroclor-induced microsomes Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 61.7, 185.2, 555.6, 1,667, and 5,000 ug/plate
Statistical Methods:	If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value is considered mutagenic. A chemical that exhibits a positive dose response over three concentration with the smallest of these increases equal to twice the solvent control is considered to be mutagenic.
Results:	Positive for TA1535 without activation.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate produced a positive mutagenic response in one of five *S. typhimurium* tester strains (TA1535) in the absence of metabolic activation.
Data Quality:	1b.

Reference:	Farrow M G (1983b); *Salmonella typhimurium*/Mammalian Microsome Plate Incorporation Assay with Compound FMC 10242; Hazleton Laboratories America Inc, Vienna, Virginia, USA; Unpublished Report No: A83-907; 2 December 1983.

5.2.12 SOURCE#12

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98.3%)
Guideline:	OPPTS 870.5100
Type:	Bacterial reverse mutation assay
System of Testing:	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537, TA 1538
GLP:	No. Quality Assurance Audit of the study confirmed that the report was an accurate representation of the data. The S-9 mix was prepared immediately before its use in the mutagenicity assay.
Remarks:	Procedure: the test material was solubilized in DMSO and diluted immediately prior to the assay Media: minimal top agar with histidine and biotin No. replicates: 3
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	0, 117, 350, 1,048, 3,145 and 9,436 ug/plate
Statistical Methods:	If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value is considered mutagenic. A chemical that exhibits a positive dose response over three concentration with the smallest of these increases equal to twice the solvent control is considered to be mutagenic.
Results:	The test material exhibited a three-fold increase at 3145 and 1048 μg/plate dose levels with strain TA153S in the nonactivated phase of testing. At the remaining lower doses, two-fold or greater increases were not observed with this strain.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate produced a positive mutagenic response in one of five *S. typhimurium* tester strains (TA1535) in the absence of metabolic activation.
Data Quality:	1b.
Reference:	Farrow M G (1983c); *Salmonella/Typhimurium*/Mammalian Microsome Plate Incorporation Assay with Compound FMC 10242; Hazleton Laboratories America Inc, Vienna, Virginia, USA; Unpublished Report no: A83-909; 5 December 1983.

5.2.13 SOURCE#13

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5550
Type:	Unscheduled DNA Synthesis in Mammalian Cells in Culture
System of Testing:	Primary rat liver cell cultures
GLP:	No
Year:	1983
Concentrations Tested:	1, 5, 10, 50, 100 ug/ml

Statistical Methods:	Mean and standard deviation of net nuclear grain counts were determined. Results were considered positive if the mean net nuclear grain count at a dose level was increased by at least five counts over the control. A test article will be judged positive if it induces a dose-related response and at least two successive doses exhibit a significant increase in the average net nuclear grains when compared to the negative controls. In the absence of a dose response, the test article should show a significant increase in the mean net nuclear grain counts in at least 3 dose levels. If a test article showed a significant increase in the net nuclear grain count at one of two dose levels without an dose response, the test article will be considered to have a marginal positive activity. The test article will be considered negative if it did not cause a significant increase in the mean net nuclear grain counts at any dose level.
Results:	Negative. None of the test doses caused a significant increase in the mean net nuclear counts, and an indication of dose response was not observed with this test article.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not mutagenic in any of the five tester strains of *S. typhimurium* in the presence or absence of metabolic activation.
Data Quality:	1b
Reference:	Thilagar A (1983a); Unscheduled DNA Synthesis in Rat Primary Hepatocytes; Microbiological Associates, Rockville, Maryland, USA; unpublished report no: A83-969; 26 September 1983.

5.2.14 SOURCE#14

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5900
Type:	*In Vitro* Sister Chromatid Exchange Assay
System of Testing:	Chinese Hamster Ovary Cells
GLP:	No
Remarks	Expression period: 26 – 30 hours in the presence of BrdU (24 hours for nonactivated assay). Time of metaphase arrest: 3 – 6 hours before being harvested. a second harvest six hours after the addition of colcemid to account for the mitotic delay. Staining: Giemsa for 5 – 8 minutes. Scoring: SCE's were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. Three doses from the activated system and four doses from the non-activated system were scored. Dose levels 2,500, 1,250 and 625 μg/ml from the activated system and 200 μg/ml from the non-activated system were not scored due to high cytotoxicity.
Year:	1983
Concentrations Tested:	2,500, 1,250, 625, 312.5, 156.25 and 78.125 μg/ml in the activated system. 200, 100, 50, 25, 12.5 and 6.25 μg/ml in the non-activated system
Statistical Methods:	Mean and standard deviation for each duplicate culture were calculated. A pairwise T-test was performed to determine the P value. Criteria for a positive test: A minimum of two dose levels with double the numberof SCEs as is observed in the negative or solvent controls. Or, a positive dose response to a number of SCEs produced over a minimum of three of the doses. If the t-test P<0.5, then the compound is considered to be positive.
Results:	Negative.

Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not induce an increased frequency of sister chromatid exchanges in CHO cells *in vitro* and can be considered nongenotoxic in this assay.
Data Quality:	1b
Reference:	Thilagar A (1983b); Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells; Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-1095; 8 November 1983.

5.2.15 SOURCE#15

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%).
Guideline:	OPPTS 870.5900
Type:	*In Vitro* Sister Chromatid Exchange Assay
System of Testing:	Chinese Hamster Ovary Cells
GLP:	No
Remarks	Expression period: 26 – 30 hours in the presence of BrdU (24 hours for nonactivated assay). Time of metaphase arrest: 3 – 6 hours before being harvested. a second harvest six hours after the addition of colcemid to account for the mitotic delay. Staining: Giemsa for 5 – 8 minutes. Scoring: SCE's were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. Three doses from the activated system and four doses from the non-activated system were scored. Dose levels 2,500, 1,250 and 625 μg/ml from the activated system and 200 μg/ml from the non-activated system were not scored due to high cytotoxicity.
Year:	1983
Concentrations Tested:	2,500, 1,250, 625, 312.5, 156.25 and 78.125 μg/ml in the activated system. 200, 100, 50, 25, 12.5 and 6.25 μg/ml in the non-activated system
Statistical Methods:	Mean and standard deviation for each duplicate culture were calculated. A pairwise T-test was performed to determine the P value. Criteria for a positive test: A minimum of two dose levels with double the numberof SCEs as is observed in the negative or solvent controls. Or, a positive dose response to a number of SCEs produced over a minimum of three of the doses. If the t-test P<0.5, then the compound is considered to be positive.
Results:	Positive. The results of the assay indicate that under the conditions of the test, the test article did cause a significant increase in the frequencies of sister chromatid exchanges in the Chinese hamster ovary cells and should be considered positive in the system.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate induced an increased frequency of sister chromatid exchanges in CHO cells *in vitro* and can be considered genotoxic in this assay.
Data Quality:	1b
Reference:	Thilagar A (1983c); Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells; Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-1097; 8 November 1983.

5.2.16 SOURCE#16

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%)
Guideline:	OPPTS 870.5375
Type:	*In vitro* Mammalian Chromosome Aberration Test
System of Testing:	Chinese Hamster Ovary Cells
GLP:	No
Remarks	Expression period: 26 – 30 hours in the presence of BrdU (24 hours for nonactivated assay). Time of metaphase arrest: 3 – 6 hours before being harvested. a second harvest six hours after the addition of colcemid to account for the mitotic delay. Staining: Giemsa for 5 – 8 minutes. Scoring: SCE's were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. Three doses from the activated system and four doses from the non-activated system were scored. Dose levels 2,500, 1,250 and 625 μg/ml from the activated system and 200 μg/ml from the non-activated system were not scored due to high cytotoxicity.
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Year:	1983
Concentrations Tested:	2500, 1250, 625, 312.5, 156.25 and 78.125 μg/ml in the activated system 2000, 1000, 500, 100, 50 and 2.5 μg/ml in the non-activated system.
Statistical Methods:	The frequency of aberrations per cell, and the frequency of structurally aberrant cells are calculated. Chromatid and chromosome gaps are presented in the data but will not be included in the average number of aberrations per cell. If a test article causes more than four times the number of cells with aberrations than the solvent control, the result will be considered significant. In the absence of a four-fold increase, a Chi-square analysis using a 2 x 2 contingency table will be used to ascertain significant differences between the number of cells with aberrations in the treatment and solvent control groups. If evidence of dose response is observed, the results of the chromosome aberration assay will be investigated for evidence of a significant ($p<0.05$) positive linear does response trend, by performing the T-test for linear regression. A positive linear dose response tread is significant if $p < 0.05$.
Results:	Compared to the number of cells with chromosome aberrations in the solvent control, none of the test doses showed a significant increase in the frequency of cells with chromosome aberrations in the activated or non-activated systems.[2] Since there was no significant positive response observed, the results of the chromosome aberration assay were not investigated for evidence of a significant positive linear dose response trend.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not cause a significant increase in the frequencies of chromosome aberrations in the Chinese hamster ovary cells and is nongenotoxic in this test.
Data Quality:	1b
Reference:	Thilagar A (1983d); Chromosome Aberrations in Chinese Hamster Ovary Cells; Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-1096; 8 November 1983.

5.2.17 SOURCE#17

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5300
Type:	*In vitro* mammalian cell gene mutation test.
System of Testing:	L5178 TK+/- Mouse Lymphoma cells
Remarks:	Total growth: non-activated - 1% to 102%. activated - 4% to 92%
GLP:	No
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.
Concentrations Tested:	134, 178, 238,317, 422, 563, 751, 1001, 1335, 1780 ug/ml with activation 24, 32, 42, 56, 75, 100, 133, 178, 237, 316 ug/ml without activation
Statistical Methods:	The test is considered to be positive if there is a positive dose response and one or more of the three highest doses exhibit a mutant frequency which is two-fold greater than the background level. The results are judged equivocal if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background. The results are negative if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.
Results:	Six non-activated and three S-9 activated cultures that were cloned exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent controls. In both the non-activated and the S-9 activated cultures a dose-dependent response was noted.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was mutagenic in mouse lymphoma cells *in vitro* with and without metabolic activation.
Data Quality:	1b
Reference:	Kirby P E (1983a); L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay; Microbiological Associates, Rockville, Maryland, USA; Unpublished Report No: A83-1064; 2 November 1983.

5.2.18 SOURCE#18

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%)
Guideline:	OPPTS 870.5300
Type:	*In vitro* mammalian cell gene mutation test.
System of Testing:	L5178 TK+/- Mouse Lymphoma cells
Method:	The nonactivated cultures that were cloned were treated with a range of test article concentrations which produced from 1% to 114% Total Growth in the first assay and from 1% to 97% Total Growth in the second assay. The S-9 activated cultures that were cloned were treated with a range of test article concentrations which produced from 4% to 93% Total Growth in the first assay and from 3% to 84% Total Growth in the second assay.
Remarks:	Total growth: non-activated - 1% to 114%. activated - 4% to 93%
GLP:	No
Year:	1983
Metabolic Activation:	With and without metabolic activation. Liver S-9 fraction from Aroclor 1254 pre-treated Sprague-Dawley rats.

Concentrations Tested:	134, 178, 238,317, 422, 563, 751, 1001, 1335, 1780 2373ug/ml with activation 16, 21, 28, 28, 38, 50, 67, 89, 119 158, 211 ug/ml without activation
Statistical Methods:	The test is considered to be positive if there is a positive dose response and one or more of the three highest doses exhibit a mutant frequency which is two-fold greater than the background level. The results are judged equivocal if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background. The results are negative if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was mutagenic in mouse lymphoma cells *in vitro* with and without metabolic activation.
Results:	Five of the nonactivated cultures that were cloned in the first assay and four of the nonactivated cultures that were cloned in the second assay exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent controls. One of the S-9 activated cultures that was cloned in the first assay and two of the S-9 activated cultures that were cloned in the second assay exhibited mutant frequencies which were more than twice the mean mutant frequency of the solvent controls. The increases in mutant frequency in the S-9 activated cultures are not considered significant since the Total Growth of these cultures in each case was less than 10%.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was mutagenic in mouse lymphoma cells *in vitro* in the absence of metabolic activation, but was not mutagenic in the presence of metabolic activation.
Data Quality:	1b
Reference:	Kirby P E (1983b); L5178Y $TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay; Microbiological Associates, Rockville, Maryland, USA; unpublished report no: A83-962; 23 August 1983.

5.3 GENETIC TOXICITY IN VIVO

5.3.1 SOURCE#1

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (98%)
Guidelines:	OPPTS 870.5385
Type:	Mammalian bone marrow chromosome aberration test
Species/strain:	Sprague-Dawley Rats
Remarks:	Dose volume: 5 ml/kg body weight/day. Number and sex of animals: 5 males per dose level A minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements, and ploidy (1 – 3). The proportion of cells in mitosis was determined for a minimum of 500 cells. The ratio of the number of cells in mitosis per total number of cells counted x 100 is defined as the mitotic index. Change in timing of dose administration: One rat in the high dose group died after a single dose administration. Toxic clinical signs included convulsions and ataxia in the high dose group. In order to reduce the severity of the convulsions, the high dose treatment was administered in two treatments separated by 1 hour on days 2 – 4. A single treatment was administered on the last dosing day in order to perform the scheduled sacrifice at 6 hours after dose administration.

	One of the two additional rats assigned to the high dose group was substituted for the rat which died as a result of dose administration.
GLP:	No
Year:	1983
Sex:	Male
Route of administration:	Oral gavage
Vehicle:	Corn oil
Doses:	0, 0.6 and 2.6 mg/kg bw/day
Exposure period:	5 consecutive days
Statistical Methods:	The percentage of damaged cells in the total population of cells scored was calculated for each treatment group. Chromatid and chromosome gaps are presented in the data but not included in the total percentage of cells with one or more aberrations. Chi-square analysis using a 2 x 2 contingency table was used to ascertain significant differences between the number of cells with aberrations in the treatment and control groups. The severity of damage within the cells is reported as the number of aberrations per cell for each treatment group. The t-test was used to compare pairwise the number of aberrations per cell for each treatment group with that of the vehicle control. Each comparison was considered to be between two independent, random samples of unequal variance and a significant increase in the treatment mean relative to the vehicle control (one-sided) was sought. A response is positive if the percentage of cells with aberrations in any treatment group is significantly increased ($p<0.05$) using Chi-square analysis and the number of aberrations per cell is also significantly increased ($p<0.05$) relative to the vehicle control group using t-test statistics.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not induce an increased incidence of chromosome aberrations in rats following oral doses of up to 2.6 mg/kg/day for 5 consecutive days and did not affect mitotic index. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was not genotoxic in this test.
Data Quality:	1b
Reference:	Putman D L (1983a); Activity of FMC 10242 (T1982) in the *In vivo* Cytogenetics Assay in Sprague-Dawley Rats; Microbiological Associates, Bethesda, Maryland, USA; Unpublished Report No: A83-972; 12 September 1983.

5.3.2 SOURCE#2

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (96%).
Guidelines:	OPPTS 870.5385
Type:	Mammalian bone marrow chromosome aberration test
Remarks:	Dose volume: 5 ml/kg body weight/day. Number and sex of animals: 5 males per dose level A minimum of 50 metaphase cells from each animal were examined and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements, and ploidy (1 – 3). The proportion of cells in mitosis was determined for a minimum of 500 cells. The ratio of the number of cells in mitosis per total number of cells counted x 100 is defined as the mitotic index.

	Change in timing of dose administration: In order to reduce the toxicity, the high dose treatment was administered in two treatments separated by 30 minutes on days 1 – 4. A single treatment was administered on the last dosing day in order to perform the scheduled sacrifice at 6 hours after dose administration.
GLP:	No
Year:	1983
Species/strain:	Sprague-Dawley Rats
Sex:	Male
Route of administration:	Oral gavage
Vehicle:	Corn oil
Doses:	0, 1, 6 or 10 mg/kg bw/day.
Exposure period:	5 consecutive days
Statistical Methods:	The percentage of damaged cells in the total population of cells scored was calculated for each treatment group. Chromatid and chromosome gaps are presented in the data but not included in the total percentage of cells with one or more aberrations. Chi-square analysis using a 2 x 2 contingency table was used to ascertain significant differences between the number of cells with aberrations in the treatment and control groups. The severity of damage within the cells is reported as the number of aberrations per cell for each treatment group. The t-test was used to compare pairwise the number of aberrations per cell for each treatment group with that of the vehicle control. Each comparison was considered to be between two independent, random samples of unequal variance and a significant increase in the treatment mean relative to the vehicle control (one-sided) was sought. The test article is considered to induce a positive response if the percentage of cells with aberrations in any treatment group is significantly increased ($p<0.05$) relative to the vehicle control group using Chi-square analysis and the number of aberrations per cell is also significantly increased ($p<0.05$) relative to the vehicle control group using t-test statistics.
Results:	There was no apparent change in ploidy or the mitotic index. The percentage of damaged cells in the total population of cells scored was calculated for each treatment group. The incidence of aberrations was not increased in the 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate treated animals. The number of aberrations per cell was also unaffected.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not induce an increased incidence of chromosome aberrations in rats following oral doses of up to 2.6 mg/kg/day for 5 consecutive days and did not affect mitotic index.. 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate was nongenotoxic in this test.
Data Quality:	1a.
Reference:	Putman D L (1983b); Activity of FMC 10242, E2915-100A (A83-1065) in the Subchronic *In Vivo* Cytogenetics Assay in Male Rats; Microbiological Associates, Bethesda, Maryland, USA; Unpublished Report No: A83-1065; 9 November 1983.

5.3.3 SOURCE#3

Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%).
Guideline:	OPPTS 870.5275
Type:	Sex-linked recessive lethal test in *Drosophila melanogaster.*

Species/strain:	*Drosophila melanogaster,* Canton S wild type males mated with females of the balancer stock "Basc", whose X chromosomes carry inversions and are marked with genes for apricot eye (wa) and Bar eye (B).
Remarks:	The compound was found to be highly toxic in range-finding tests, necessitating both a low concentration of the chemical and a reduced exposure time. Adult males (2 – 3 days old and previously starved for 4 hours) were treated in groups of 15 in shell vials (23 mm by 90 mm) plugged with rayon fibre balls. The base of each vial was covered with a disc of glass fibre filter material on which 450 μl of feeding solution was pipetted. The standard procedure is to transfer the males daily for 3 days to new treatment vials with freshly-prepared feeding solution. Males were untreated for the first two days of the test period. Exposure was stopped at approximately 22 hours, due to high toxicity. Negative control flies were fed by the standard procedure on 5% sucrose in 10% ethanol, buffered to pH 6.8. The exposures were performed at room temperature (approximately 22°C) with no control over the photoperiod.
GLP:	No
Year:	1983
Route of administration:	Feeding solution
Vehicle:	Sucrose/ethanol feeding solution
Doses:	7.5 ppm
Exposure period:	22 hours
Statistical Methods:	The overall mutation frequency of the treated group was compared with the overall mutation frequency of the concurrent negative controls by applying the "Normal Test". When an individual P1 male produces more than 1 lethal among his progeny, the group of lethals can be called a multiple. A multiple may result from many independent mutations in the post-meiotic germ cells or from a single spontaneous mutation in a gonial cell which then multiplies to produce a "cluster" of sperm cells carrying identical recessive mutations. A statistical test based on cumulative Poisson distribution is used to determine whether or not the multiple could be considered a "cluster".
Results:	For 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate there were 8 lethals in 6944 X chromosomes tested (0.115%). The negative control value was 0.075% and the positive control (DMN) yielded 7.29%.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate does not induce mutations in *Drosophila melanogaster* when administered to male flies orally at 7.5 ppm in feeding solution.
Data Quality:	1b.
Reference:	Valencia R (1983); *Drosophila* Sex-Linked Recessive Lethal Assay of 2,3-Dihydro-2,2-Dimethyl-7-Benzofuranyl N-Methyl Carbamate (Carbofuran, T-2047); University of Wisconsin, Madison, Wisconsin, USA; Unpublished Report No: A83-1019; 7 November 1983.

5.3.4 SOURCE#4

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (97.6%)
Guideline:	OPPTS 870.5275 Sex-linked recessive lethal test in *Drosophila melanogaster.*
Type:	Sex-linked recessive lethal test in *Drosophila melanogaster.*
Remarks:	Toxicity test: Dose levels were selected based on the results of toxicity tests with doses of 1.0, 2.5, 5.0, 10.0, 20.0 and 50.0 μg/ml. By the end of the 24 hour dosing period, all flies at the 20 and 50 μg/ml doses were dead. Flies from the remaining four doses, and the solvent controls, were mated to virgin female flies to assess the fertility of

	the treated males. Fertility at 10 μg/ml, and 5 μg/ml was considered sufficient to conduct the sex-linked recessive lethal assay. Adjustment for low fertility: Due to low fertility in the sex-linked recessive lethal assay, lower than expected numbers of F1 flies available for pair mating. To produce the necessary numbers of scored chromosomes, this assay was done in two runs, each with its own negative and positive controls.
GLP:	No
Year:	1983
Species/strain:	*Drosophila melanogaster*
Sex:	males
Route of administration:	Oral - feeding from a glass filter paper saturated with sucrose solution containing the test material
Vehicle:	1% sucrose solution
Doses:	0, 5 and 10 ug/ml
Exposure period:	24 hours
Statistical Methods:	The overall mutation frequency of the treated group was compared with the overall mutation frequency of the concurrent negative controls by applying the "Normal Test". When an individual P1 male produces more than 1 lethal among his progeny, the group of lethals can be called a multiple. A multiple may result from many independent mutations in the post-meiotic germ cells or from a single spontaneous mutation in a gonial cell which then multiplies to produce a "cluster" of sperm cells carrying identical recessive mutations. If a cluster cannot be attributed to handling or treatment, then the cluster was determined to have arisen from a spontaneous mutation in a gonial cell, which then replicated, and the cluster was counted as single event to avoid biasing the interpretation of results. A positive mutagenic effect is concluded if the difference between a concurrent control group is statistically significant at the 5% level according to the Kastenbaum-Bowman test. The test is considered negative if the following two criteria are met. 1. The increase in the treated group over the control is less than 0.2% and the sample size is large enough to allow the detection of a statistically significant increase of 0.2% based on the Kastenbaum-Bowman test. 2. If none of the broods analysed shows a positive result, the increase in the treated group of at least two broods must be less than 0.4% and the sample size in each of the two broods must be large enough to allow the detection of a statistically significant increase of .4%
Results:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not produce increased frequencies of sex-linked recessive lethal mutations and is not considered mutagenic in this assay.
Conclusion:	Negative.
Data Quality:	1b
Reference:	DeGraff W G (1983b); Mutagenicity Evaluation of FMC 10242 Lot No. RHB-11 FMC Study No.A83-1060 for the Sex-Linked Recessive Lethal Test in *Drosophila melanogaster* – Final Report; Litton Bionetics Inc, Kensington, Maryland, USA; Unpublished Report No: A83-1060; November 1983.

5.3. 5 SOURCE#5

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (99%)
Method:	OECD 474
Type:	*In vivo* Mouse Micronucleus study

Species/Strain:	Crl:CFW SW(BR) Swiss Webster mice
GLP:	Yes.
Remarks:	
Year:	2004
Concentrations Tested:	0, 1.25, 2.5, and 5.0 mg/kg in corn oil.
Statistical Methods:	Analysis of variance on untransformed proportions of cells with micronuclei per animal and on untransformed PCE:NCE rations when the variances were homogeneous. Ranked proportions were used for heterogeneous variance. If the analysis of variance was statistically significant, Dunnett's t-test was used to determine which dose groups, if any, were statistically significantly different from the vehicle control. Analyses were performed separately for each sampling time. The low, mid, and high-dose groups, as well as the positive control group, were compared with the vehicle control group at the 5%, one-tailed probability level.
Results:	Negative.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not increase the incidence of micronuclei in mice dosed with up to 5 mg/kg and is considered non-genotoxic in this test.
Data Quality:	1a.
Reference:	Erexson, G L (2004); *In vivo* Mouse Micronucleus Assay with Carbofuran Technical, Covance Laboratories Inc. Vienna, Virginia, USA; Unpublished Report No: A2004-5751; Draft report date: 22 December 2004.

5.3.6 SOURCE#6

Test Substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (99%)
Method:	OECD 475
Type:	*In vivo* Chromosomal Aberratoin study
Species/Strain:	Crl:CFW SW(BR) Swiss Webster mice
GLP:	Yes.
Remarks:	Three of 18 mice at 5.0 mg/kg died. Clinical signs of toxicity included circling to the left, hypoactivity, irregular respiration, recumbency and/or tremors.
Year:	2004
Concentrations Tested:	0, 1.25, 2.5, and 5.0 mg/kg in corn oil.
Statistical Methods:	Ranked analysis (non-parametric) for heterogeneity and trend where applicable. Gaps were not counted as significant aberrations. Open breaks were considered as indicators of genetic damage, as were configurations resulting form the repair of breaks. Individual animal results and a summary by dose group for structural chromosomal aberrations, polyploidy, and endoreduplication data were tablulated. Comparisons to the vehicle control group were analyzed by ranked analysis of variance procedures. Criteria for a positive response are a statistically significant, dose-related increase in the number of structural aberrations for at least one dose level.
Results:	Negative.
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not increase the incidence of chromosome aberrations in mice dosed with up to 5 mg/kg and is considered non-genotoxic in this test.
Data Quality:	1a.

Reference:	Erexson, G L (2004); Chromosomal Aberrations *in vivo* in Mouse Bone Marrow Cells with Carbofuran Technical, Covance Laboratories Inc. Vienna, Virginia, USA; Unpublished Report No: A2004-5750; Draft report date: 22 December 2004.

5.4 REPEATED DOSE TOXICITY: CHRONIC STUDIES

5.4.1 SOURCE #1

Type:	Chronic toxicity/carcinogenicity
Species:	rat
Sex:	male/female
Strain:	Charles River CD
Route of admin.:	Oral feed
Exposure period:	2 years
Frequency of treatm.:	continuous
Post exposure period:	no
Doses:	0, 10, 20, 100 ppm
Control group:	yes
NOAEL:	20 ppm (1 mg/kg/day) based on plasma, erythrocyte and brain cholinesterase inhibition
Method:	OPPTS 870.4300, OECD 453
Year:	1979
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot no. MRS1314, purity 95.6 %
Method:	TEST ORGANISMS - AGE AT STUDY INITIATION: weanling Weight at study initiation 76 -143 g (males), 68 -134 g (females) - Number of animals: 90/sex/treatment. Animals were individually identified with ear tags. Exposure period lasted up to 2 years CLINICAL OBSERVATIONS AND FREQUENCY: - Clinical signs: twice daily for signs of overt toxicity, moribundity and mortality. Detailed clinical observations and recording of palpable masses were conducted weekly. - Body weight and Food consumption: weekly - Ophthalmoscopic examination: pre-test and at 12 and 24 months - Hematology, clinical chemistry, including RBC, plasma and brain cholinesterase activity, and urinalysis were conducted at 6, 12 and 18 months on 10 animals/sex/group and on 20 animals/sex/group at 24 months. -Gross necropsies and histopathology: 10 rats/sex/group at 6, 12, and 18 months and on all survivors at end of study. ORGANS EXAMINED AT NECROPSY AND PATHOLOGY: - Organ weights: spleen, liver, kidneys, orary/testis with epididymis, heart, brain, lungs, adrenals, thyroid/parathyroid and pituitary. - Macroscopic: complete on all animals - Microscopic: masses and gross lesions of indefinite nature were collected and individually identified from all necropsied animals. Microscopic evaluation of brain, spinal cord, pituitary, thyroid, thymus, adrenals, heart, lungs, spleen, liver, kidneys, esophagus, stomach, duodenum, ileum, cecum, colon, pancreas, ovary, uterus, testes with epididymis, prostate, seminal vesicle, salivary gland, lymph node, urinary bladder, sciatic nerve with muscle, eye, bone marrow, sternum, mammary gland, skin and bone marrow.

	ANALYSES: Diets were analyzed every 3 months by gas chromatography. STATISTICAL METHODS: Survival of groups will be compared using life-table methods. Tumor incidence among groups will be made using life-table methods and Fisher's exact or Chi-square test. Analysis of body weights and organ weights, food consumption and clinical laboratory measurements will be performed using analysis of variance.
Result	TOXIC RESPONSE/EFFECTS: - No treatment-related differences were observed in appearance, behavior, food consumption or ophthalmic, hematologic or urinalysis values. At 100 ppm, mean body weights of male rats were significantly decreased compared to controls throughout the study (ca. 10%); female mean body weights were significantly decreased at week 104 (9%). At 100 ppm, plasma, erythrocyte and brain cholinesterase activities were inhibited in males by 37%, 24% and 25%, respectively, and in females by 26%, 19% and 43%, respectively. No treatment-related gross lesions were observed at necropsy; histopathologic examination of tissues did not show any treatment-related changes in the incidences of neoplastic or non-neoplastic lesions. . Chemical analysis: Measured concentrations in test diets were from 76 to 131% of target concentrations. STATISTICAL RESULTS: The effects on body weight (gain) at 100 ppm (males); depression of RBC, plasma and brain cholinesterase at 100 ppm. Sporadic organ weight changes were not consistent across dose groups and were not associated with any histopathology; therefore, the biological significance is unknown.
Test substance	CAS 1563-66-2, purity 95.6%.
Conclusion	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical was not oncogenic in the rat. The NOEL for toxicity was 20 ppm (1 mg/kg/day) based on plasma, erythrocyte and brain cholinesterase depression and body weight depression.
Reliability	(2e)
Remarks	Age of animals at study initiation was younger than guideline requirement (8 wk); adequate survival at 24 months (>25%); adequate analyses of diet stability/homogeneity; study is adequate for assessment.
Source	Goldenthal E I (1979a); Two-Year Dietary Toxicity and Carcinogenicity Study in Rats; IRDC, Mattawan, Michigan, USA; Unpublished Report No: ACT 130.51; 18 December 1979.

5.4.2 SOURCE #2

Type:	Chronic toxicity/carcinogenicity
Species:	Mice
Sex:	male/female
Strain:	Charles River CD –1
Route of admin.:	Oral feed
Exposure period:	2 years
Frequency of treatm.:	Continuous
Post exposure period:	No
Doses:	0, 20,125, 500 ppm
Control group:	Yes
NOAEL:	20 ppm (2.8mg/kg bw/day)
Method:	OPPTS 870.4300, OECD 453
Year:	1980
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot no. MRS1314, purity 95.6 %

Method:

TEST ORGANISMS
- Age at study initiation: weanling
- Weight at study initiation 20 – 37 g (males), 18 - 29 g (females)
- Number of animals: 100/sex/group

CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs and Mortality: at least twice daily during the treatment period
- Body weight and Food consumption: weekly
- Hematology, biochemical and urinalysis: during week 6, 12 and 18 on 10 animals/sex/group and on 20 animals/sex/group at 24 months. Brain cholinesterase activity was determined on animals selected for clinical laboratory determinations.

ORGANS EXAMINED AT NECROPSY AND PATHOLOGY-
- -Organ weights: spleen, liver, kidneys, gonads, heart, brain, lungs, adrenals, thyroid and pituitary.

-Gross necropsies and histopathology: 10 mice/sex/group at 6, 12, and 18 months and on all survivors at 24 months- Macroscopic: complete on all animals- Microscopic: masses and gross lesions of indefinite nature were collected and individually identified from all necropsied animals.

DIET ANALYSES:
-Method: Gas chromatographic analysis of diets on the day of preparation for weeks 1, 5, 14, 26, 40, 53, 66, 79, 92 and 104.

STATISTICAL ANALYSES: ANOVA (one-way clasification) was used for body weights, food consumption, clinical chemistry/hematology/urinalysis /cholinesterase data and organ weight data. Bartlett's test for homgeneity of variances and the appropriate t-test (for equal or unequal variances) using Dunnett's multiple comparison tables were used to judge significance of differences.

Result:

TOXIC RESPONSE/EFFECTS
- There were no significant differences noted in mortality or clinical signs that were attributed to the test material treatment. The mean body weights and food consumption were slightly lower for male and female mice at 500 ppm relative to controls (ca. 4-5%). Mean hematologic,biochemical and urinalysis values were similar to control mice. Brain cholinesterase activity was depressed at the 125 and 500 ppm levels. There were no significant organ weight changes or histopathological findings.

Dietary analyses: Average recoveries were 90, 93, and 95% at the 20, 125 and 500 ppm levels respectively.

STATISTICAL RESULTS: Brain cholinesterase values were significantly different at all intervals at 500 ppm (27 – 52% inhibition, males; 41-55% inhibition, females) and most of the intervals at 125 ppm (16-31% inhibition, males; 20-30 % inhibition, females). No treatment-related lesions were observed at necropsy or following histopathologic examination of the tissues.

Test substance: CASNo. 1563-66-2, purity 95.6%.

Conclusion: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate technical was not oncogenic in the mouse. The NOEL was 20 ppm (3 mg/kg/day) based on brain cholinesterase depression.

Reliability: (2e)

Remarks: Age of animals at study initiation was younger than guideline requirement (8 wk); adequate survival at 24 months (>25%); adequate analyses of diet stability/homogeneity; study adequate for assessment.

Source: Goldenthal E I (1980); 2 Year Dietary Toxicity and Carcinogenicity Study in Mice; IRDC, Mattawan, Michigan, USA; Unpublished Report No: ACT 150.52; 4 January 1980.

5.5 REPRODUCTIVE TOXICITY

Type:	Three Generational Reproduction
Species:	Rat
Sex:	Male/female
Strain:	Charles River CD
Route of admin.:	oral feed
Exposure period:	3 generations
Frequency of treatm.:	Continuous
Post exposure period:	No
Doses:	0, 20, 100 ppm
Control group:	Yes
NOAEL:	20 ppm (1.2 mg/kg bw/day)
Method:	Similar to OPPTS 870.3800, OECD 416
Year:	1979
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot number MRS 1314, purity 95.6%
Method:	TEST ORGANISMS - Age: rats were 39 days at start of treatment and 100 days at mating. - Weight at study initiation: 145 – 206 g (males), 108 – 172 g (females) - Number of animals: 1st and 2nd generations each had 10 male and 20 female parental rats per dose group; 3rd generation parents numbered 12 males and 24 females per dose group. DIET PREPARATION AND ANALYSES: Test diets were prepared by mixing test article with acetone and adding to basal diet in a premix. The premix was blended with additional diet to give homogenous diets. The control group had acetone at the same concentration added to basal diet. Diets were prepared fresh weekly.Diets were collected and analyzed on study weeks 1, 5, 27, 40, 52, 75 and 87. DOSING SCHEDULE: Animals were exposed to control or treated diets during a pre-mating period of about 61 days, during mating, gestation, lactation, for three generations with two litters per generation. F1 and F2 parental animals were exposed to control or treated diets after weaning. MATING PROCEDURES: One male and two females from the same treatment group were housed together for 15 days of mating (P1 parental animals). Upon confirmation of pregnancy by daily vaginal smears or copulatory plug, gestation day 0 was designated. Lactation day 0 was defined as the day all pups in a litter were found. After weaning, the pups designated as F1a pups, were sacrificed. Following a 10 day rest period, the P1 parental females were mated a second time with different males (two females to one male) within the same treatment group to produce the F1b litters. After weaning, 10 male and 20 females F1b pups were randomly selected from each treatment group to comprise the second generation parental rats (F1). All surviving P1 parental rats were sacrificed. The designated F1b pups were maintained on conrol or treated diets for 30 weeks. At 100 days of age the F1 parental rats were mated to produce the F2a litters. Mating procedures were the same as described for the P1 parental generation with avoidance of brother-sister matings. After weaning, the F2a pups were sacrificed. Following a ten-day rest period, the F1 parental ras were mated a second time to produce the F2b litters using the same mating procedure as the F2a litter. One week after weaning, 12 male and 24 female F2b pups were randomly selected as the third generation parental rats (F2). Twenty male and female F2b pups were selected randomly for necropsy. All remaining F2b and F1 animals were sacrificed. The designated F2 parental animals were fed treated or control diets through week 29 of this generation. At about

100 days of age, the F2 parental rats were mated to produce the F3a litters. The mating procedure was the same as described above. Brother-sister matings were avoided. After weaning the F3a litters were sacrificed. Following a ten-day rest period after weaning the F3a litters, the F2 parents were mated a second time by the same procedure to produce the F3b litters.
PUP EXAMINATIONS: Upon delivery, all pups from both litters of each generation were examined for external abnormalities, counted, sexed and weighed at designated intervals during lactation. During the F1 and F2 generations, the numbers of male and female pups at lactation days 0, 4, 14 and 21 were recorded. Litter size was reduced to ten pups of equal sex ratio, if possible, on lactation day 4. Organ weights were measured among the F3b weanlings at terminal sacrifice for spleen, liver, kidneys, ovaries, testes, heart, adrenals, thyroid.
CLINICAL OBSERVATIONS: Parental rats (P1, F1 and F2) and pups (F1a, F1b, F2a, F2b, F3a, F3b) were observed daily for clinical signs of toxicity and mortality. Detailed observations were recorded weekly for parental animals.
BODY WEIGHT / FOOD CONSUMPTION MEASUREMENTS Individual body weights and food consumption were recorded weekly for the parental animals. In addition, body weights for parental females of the F1 and F2 generations were measured on gestation days 0, 7, 14 and 21 and lactation days 0, 4, 14, and 21. Pups were weighed on lactation days 0, 4, 14 and individually, by sex, on lactation day 21.
ORGAN WEIGHTS: Organ weights were measured on F2 parental animals and F3b weanlings at terminal sacrifice for spleen, liver, kidneys, ovaries/testes, heart, adrenals, and thyroid.
PATHOLOGY: Designated parental animals of the F1, F2b, F2 and F3b generations: tissues evaluated included brain, thyroid, parathyroid, lungs, heart, pancreas, spleen, mesenteric lymph node, ovearies, liver, kidneys, tests, epididymis, seminal vesicle, mammary gland, prostate, uterus, cervix, and all lesions. Gross necropsy was conducted on all P1, F1 and F2 parental animals and F2b and F3b pups.
REPRODUCTIVE PARAMETERS: Male and female fertility, gestation length, and the growth, viability, and survival of the pups through weaning were evaluated. Gross necropsies were performed on all parental rats and on pups from the F2b and F3b litters. Tissues from these rats were microscopically examined.
STATISTICAL ANALYSES: All statistical analyses compared treatment groups with the control group with the level of significance at $p<0.5$. Male and female fertility indices were compared using Chi-square test criterion with Yates' correction for 2x2 contingency tables and/or Fisher's exact probablity test to judge statistical significance of differences. Gestation and pup survival indices were compared by the Mann-Whitney U-test to judge the significance of differences. Parental body weights and mean numbers of live-born pups/litter were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal v ariances) using Dunnett's multiple comparison tables to judge the significance of differences. Mean body weights of the pups at lactation day 21 were compared by analysis of variance (hierarchal classification) and t-test using Dunnett's multiple comparison tables to judge the significance of differences.

Result:

TOXIC RESPONSE/EFFECTS
There were no treatment-related changes in general behavior or appearance of the parental rats and pups, except for dehydration noted in some litters of the third generation pups at 100 ppm. Mean body weights of the parental rats of both sexes in the 100 ppm group were consistently lower than the control values throughout treatment. Food consumption values (g/kg/day) for the P1, F1 and F2 males and the P1 females were generally lower than the controls throughout their treatment periods. Food consumption values for the F1 and F2 females were comparable to controls. No treatment-related differences were observed between control and treated parental rats with respect to male and female fertility and length of gestation periods. The growth, viability and

	survival of pups in the 20 ppm group was generally comparable to controls. The viability and survival of pups in the 100 ppm group was generally comparable to controls, except on lactation day 4, when the F1a, F2a and F3a litter survival was slightly reduced. Mean body weights of pups in the 100 ppm group in all generations and all litters were reduced compared to control (with statistical significance on lactation day 21). Gross necropsy, organ weights, necropsy findings and microscopic histopathologic examinations revealed no compound-related changes. Random changes in organ weights during the study were not considered biologically significant. Test diets contained 80 – 119% of target concentrations.
Test substance:	CAS 1563-66-2 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 95.6% purity
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate is not a reproductive toxicant. The NOEL was 20 ppm based on reduction in pup body weights.
Reliability:	(2e)
Remarks:	Only two dose levels were used (guideline requires three dose levels); matings used 2 females:1 male (guideline requires 1:1 mating); no evaluation of sperm motility and morphology or estrous cycle length/pattern was conducted; postnatal day 7 pup body weight was not measured; age of vaginal opening for F1 weanings selected for mating was not determined; organ weights for brain and pituitary were not determined; parental animals were chosen randomly without regard to litter (guideline requires that one male and one female from each litter be chosen randomly for the parental animals). Despite these deficiencies the study can be used for assessment.
Source:	Goldenthal E I (1979b); Three Generation Reproduction Study in Rats; IRDC, Mattawan, Michigan, USA; Unpublished Report No: ACT 131.53; 9 November 1979.

5.6 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.6.1 SOURCE #1

Type:	Developmental toxicity/Teratogenicity
Species:	Rat
Sex:	Female
Strain:	Charles River COBS CD
Route of admin.:	Dietary
Duration of the test:	Through gestation day 19
Frequency of treatm.:	Continuous during gestation days 6 - 19
Doses:	0, 20, 60, 160 ppm
Control group:	Yes
NOEL :	20 ppm (1.47mg/kg bw) for maternal toxicity 160 ppm (10.96 mg/kg bw) for developmental toxicity
Method:	Similar to US EPA OPPTS 870.3700, OECD 414
Year:	1981
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot no. MRS1314, purity 95.6%
Method:	TEST ORGANISMS - Age: 12 weeks of age (virgin females) - Weight at study initiation (mating): mean per group - 221 –351 g

- Number of animals: 20 pregnant females per treatment group
MATING: 1 female was mated with one male. Occurrence of copulation by daily inspection for a copulatory plug or by the presence of sperm in a vaginal smear indicated day 0 of gestation.
TEST ARTICLE EXPOSURE: Pregnant rats were exposed to control or treated diets on gestation days 6-19. Fresh diets were prepared fresh weekly and analyzed for test article concentration.
MATERNAL OBSERVATIONS: Parental rats were observed daily for clinical signs of toxicity and mortality. Body weight gain and food consumption were measured on gestatoin days 0, 6, 8, 10, 12, 14, 16, 18 and 20. On gestation day 20 females were sacrificed and the abdominal cavity examined. The number and location of viable/nonviable fetuses; early/late resorptions, total number of implantations and corpora lutea were recorded.
FETAL EXAMINIATIONS: Cesarean sections were performed on 20 pregnant females on gestation day 20; fetuses were individually weighed, measured (crown to rump length) and examined for external malformations/variations, including the palate and eyes. Each fetus was externally sexed and identified by tag, then dissected, internally sexed and examined for visceral malformations/ variations. The heart was dissected by a modified Staples technique. The heads of half of the fetuses were fixed in Bouin's solution for visceral examination by the Wilson technique. All fetuses (including those with heads removed) were eviscerated, fixed in alcohol, macerated in potassium hydroxide and stined with Alizarin Red S for skeletal examinations.
STATISTICAL ANALYSES: All statistical analyses compared the treated groups to the control group with a level of significance of $p<05$. The male to female sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2x2 contingency tables and/or the Fisher's exact probablity test. The number of early and late resorptions and postimplantation losses were compared by the Mann-Whitney U-test . The mean number of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by analysis of variance (one-way classifications), Bartlett's test for homogeneity of variances and the appropriate t-test using Dunnet's multiple comparison tables.
TOXIC RESPONSE/EFFECTS
No biologically significant clinical signs were attributable to treatment. Mean maternal body weights were significantly decreased in the 60 and 160 ppm groups during the first two days of treatment, resulting in a slightly reduced mean gain in the 60 ppm group and a moderately reduced gain in the 160 ppm group. Mean food consumption values for the 20 and 60 ppm groups were comparable to control, whereas food consumption for the 160 ppm group was slightly reduced. There were no significant differences in the mean number of fetuses or litters with malformations, numbers of corpora lutea, total implantations, early or late resorptions, mean postimplantation loss, fetal length, fetal weight, fetal sex distribution compared to controls. There were no increaesd incidences of any external, visceral or skeletal malformations/variations in treated animals.

Test substance: CAS 1563-66-2 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 95.6% purity

Conclusion: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate techincal was not teratogenic when administered in the diet at a level of 160 ppm on gestation days 6-19.. The developmental NOEL was 160 ppm in diet (10.96 mg/kg bw). The maternal NOEL was 20 ppm in diet (1.47 mg/kg bw).

Reliability: (2e)

Remarks: Meets guideline but not fully GLP

Flag: Critical study

Source: Rodwell D E (1981); Teratology and Postnatal Study in the Rat with Carbofuran; IRDC, Mattawan, Michigan, USA; Unpublished Report No: A80-444; 8 January 1981.

5.6.2 SOURCE #2

Type:	Developmental toxicity/Teratogenicity
Species:	Rat
Sex:	Female
Strain:	Charles River COBS CD
Route of admin.:	Oral gavage
Duration of the test:	Gestation day 20
Frequency of treatment:	Single daily dose on days 6 – 15 of gestation
Doses:	0, 0.25, 0.50, and 1.20 mg/kg/day
Control group:	Yes (corn oil vehicle at a constant volume of 7.5 ml/kg)
NOEL Maternal and NOEL Developmental:	≥1.2 mg/kg/day
Method:	Similar to OPPTS 870.3700, OECD 414
Year:	1981
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot no. MRS1314
Method:	TEST ORGANISMS - Age: 19 weeks - Weight at study initiation: 237-343 grams MATING: 1 female was mated with one male. Pregnancy was confirmed by daily inspection for a copulatory plug or the presence of sperm in a vaginal smear. The evidence of mating was designated as day 0 of gestation. Mated females were randomly assigned to four groups (one vehicle control and three treatment groups) of 25 pregnant rats/group. TREATMENT: Pregnant animals were dosed once daily on gestation days 6 through 15 by oral gavage with doses of 0, 0.25, 0.50 or 1.20 mg/kg using corn oil as the vehicle at a constant volume of 7.5 ml/kg. OBSERVATIONS: Females were observed daily for mortality and clinical signs of toxicity before treatment and twice daily for mortality and clinical signs on days 6-20 of gestation. Body weights were recorded on gestation days 0, 6, 9, 12, 15, 18 and 20. CESAREAN SECTION OBSERVATIONS: Cesarean sections were performed on all females on gestation day 20 and uteri were excised, weighed and examined. The number and location of viable and nonviable fetuses, early and late resorptions, total number of implantations and corpora lutea were recorded. Gross necropsies were performed on all females. FETAL EVALUATIONS: All fetuses were weighed, measured (crown to rump length) and examined for external malformations and variations. The heads from one-half of the fetuses were removed and placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning (Wilson technique). Each fetus was dissected and examined for visceral malformations and variations. The heart was dissected by a modified Staples method. All fetuses, including those without heads, were eviscerated, fixed in alcohol, mascerated in potassium hydroxide and stained with Alizarin Red S for skeletal examinations. STATISTICAL ANALYSIS: All statistical analyses compared the treated groups to the control group with a level of significance of $p<05$. The male to female sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2x2 contingency tables and/or the Fisher's exact probablity test. The number of early and late resorptions and postimplantation losses were compared by the Mann-Whitney U-test . The mean number of viable fetuses, total implantations, corpora lutea

	and mean fetal body weights were compared by analysis of variance (one-way classifications), Bartlett's test for homogeneity of variances and the appropriate t-test using Dunnet's multiple comparison tables.
Results:	TOXIC RESPONSE/EFFECTS: Survival was 100% in all dosage groups. There were no treatment-related biologically significant differences in appearance, behavior or mean maternal body weight gain of rats in any of the treated groups. There were no biologically meaningful or statistically significant differences in the mean numbers of *corpora lutea*, total implantations, early and late resorptions, postimplantation loss, viable fetuses, the fetal sex distribution, mean fetal body weight or the number of litters with malformations in any of the 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate treated groups when compared to the control group. Mean fetal body length and the number of litters (and fetuses) with genetic and developmental variations in the 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate treated groups were also comparable to the control group. There were no teratogenic responses to the test article at any dose level.
Test substance:	CAS 1563-66-2 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 95.6% purity
Conclusion:	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate techincal was not teratogenic at a dose of up to 1.2 mg/kg on gestation days 6-15.. The developmental and maternal NOEL was $\geq$ 2.0 mg/kg.
Reliability:	(2e) Restriction: dosing went only to gestation day 15 (should be day 19); food consumption was not recorded. This study is adequate for assessment.
Flag:	Critical study
Source:	Rodwell D E (1981); Teratology and Postnatal Study in the Rat with Carbofuran; IRDC, Mattawan, Michigan, USA; Unpublished Report No: A80-453; 26 December 1980.

5.6.3 SOURCE #3

Type:	Developmental toxicity/Teratogenicity
Species:	Rabbit
Sex:	Female
Strain:	New Zealand White
Route of admin.:	Gavage
Duration of the test:	Mating through gestation day 29
Frequency of treatm.:	Single daily dose from gestation days 6 through 18
Doses:	0, 0.12, 0.50 and 2.00 mg/kg/day in 0.5% Methocel®
Control group:	Yes, 0.5% Methocel® at a constant volume of 1 ml/kg
NOEL maternal and fetal:	$\geq$2.00 mg/kg/day
Method:	Similar to OPPTS 870.3700 and OECD 414
Year:	1980
GLP:	No
Test substance:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, Lot number MRS1314 (95.6% purity)
Method:	TEST SPECIES- Age: 7 months at initiation- Weight of mated females: 3561 – 3733 g (group means) Female New Zealand White rabbits were impregnated artificially with semen, then injected with chorionic gonadotropin to induce ovulation. The day of insemination was designated as gestation day 0. DOSING: 20 females per treatment group received a single daily dose by oral gavage on gestation days 6 through 18 at a constant volume of 1 ml/kg vehicle (0.5% Methocel®). The control group received the vehicle only (0.5% aqueous methyl cellulose) on a comparable regimen at a volume of 1 ml/kg. Caesarean

sections were performed on all surviving females on gestation day 29.
MATERNAL EXAMINATIONS: The uteri were weighed and examined for number/location of viable/nonviable fetuses, early/late resoreptions, number of total implantations and corpora lutea. Gross necrospy was performed on the abdominal and thoracic cavities and organs of the dams.
BODY WEIGHTS: Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24 and 29.
FETAL EXAMINATIONS: All fetuses were weighed, measured and examined for extermal malformations and variations, including the palate and eyes. Each fetus was dissected, internally sexed and examined for visceral malformations, including brain by mid-coronal slice. Fetuses were preserved om 10% neutral buffered formalin for histopathological evaluation, if necessary. Skeletal malformations were examined on fetuses which had been treated with potassium hydroxide and stained with Alizarin Red S.
STATISTICAL ANALYSES: All statistical analyses compared the treated groups to the control group with a level of significance of $p<.05$. The male to female sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2x2 contingency tables and/or the Fisher's exact probablity test. The number of early and late resorptions and postimplantation losses were compared by the Mann-Whitney U-test . The mean number of viable fetuses, total implantations, corpora lutea and mean fetal body weights were compared by analysis of variance (one-way classifications), Bartlett's test for homogeneity of variances and the appropriate t-test using Dunnet's multiple comparison tables.

Results: TOXIC RESPONSE/EFFECTS: Survival was 100% in the control group and in the 0.12 and 0.50 mg/kg/day dosage groups. Three dams aborted near the end of the gestation period; one each in the 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate treated groups. One dam in the 2.00 mg/kg/day dosage group died on gestation day 11; a cause of death could not be determined at necropsy. During gestation, matting and/or staining of the anogenital haircoat was noted in all groups, with an increase in duration observed in the 2.00 mg/kg/day dosage group. Mean maternal body weight gain in the 0.12 and 0.50 mg/kg/day dosage groups was comparable to the control group throughout gestation. A 20% reduction in mean maternal body weight gain was noted in the 2.00 mg/kg/day dosage group during the treatment period compared to the control group; mean weight gain, thereafter, was comparable to the control group. There were no biologically meaningful differences or statistically significant differences in mean numbers of corpora lutea, total implantations, early/late resorptions, postimplantation loss, viable fetuses, sex distribution, fetal body weight, fetal length and number of litters with malformations (or developmental and genetic variations) in any of the 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate treated groups when compared to the control group.

Conclusion: Treatment with 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate did not produce a teratogenic response when administered orally to pregnant rabbits at a dosage level of 2.00 mg/kg/day on gestation days 6-18. The developmental NOEL was $\geq$2.0 mg/kg. The maternal NOAEL was 0.5 mg/kg bw/day based on reduced body weight in dams at 2 mg/kg bw.

Reliability: 2e

Remarks: Dosing of test article should have been continued until the day before parturition. Food consumption not recorded.

Source: Laveglia J (1981); Teratology Study in the Rabbit with Carbofuran; International Research and Development Corporation, Mattawan, Michigan, USA; Unpublished Report No: A80-452; 20 April 1981.

Flag: Critical study

CRITERIA FOR RELIABILITY CODES
(Adapted from Klimisch et al 1997)

Code of Reliability	Category or reliability
1	**Reliable without restriction**
1a	GLP guideline study (OECD, EC, EPA, FDA, etc.)
1b	Comparable to guideline study
1c	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
2	**Reliable with restrictions**
2a	Guideline study without detailed documentation
2b	Guideline study with acceptable restrictions
2c	Comparable to guideline study with acceptable restrictions
2d	Test procedure in accordance with national standard methods with acceptable restrictions
2e	Study well documented, meets generally accepted scientific principles, acceptable for assessment
2f	Accepted calculation method
2g	Data from handbook or collection of data
3	**Not reliable**
3a	Documentation insufficient for assessment
3b	Significant methodological deficiencies
3c	Unsuitable test system
4	**Not assignable**
4a	Abstract
4b	Secondary literature
4c	Original reference not yet available
4d	Original reference not yet translated
4e	Documentation insufficient for assessment